Exhaled & serum biomarkers in pulmonary diseases

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What are biomarkers?
Why resort to biomarkers?

- Early diagnosis
- Differentiating diseases
  - Classic
    - Cardiogenic vs. non cardiogenic PE
    - Sepsis vs. no sepsis
- Monitoring disease activity
- Prognostication
- Monitoring response to therapy
Source

- Exhaled breath
  - FENO
  - Other Exhaled breath condensate (EBC)

- Serum
  - CRP
  - Procalcitonin
  - S-TREM 1

- Sputum / BAL

- Tissue
• Micron/ sub-micron particles emanating from mouth / ET have been identified

• Origin is of speculation
  – Sheer force of turbulence aerosolizing airway lining fluid
  – Alveolar origin – due to force applied to open alveoli- potential to kinetic conversion
Points to consider

• How to collect?
• What to collect?
• How to isolate?
• Contribution with respect to particle size
• Standardization in disease & health
• Dilution factor
• Contamination factor
NO synthesis

\[ \text{NOS} \quad \text{NADPH} \quad \text{BH4} \quad \text{FAD} \]

\[ \text{O}^\cdot \quad \text{NO}^\cdot \]

\[ \text{L-arginine} \quad \text{L-citrulline} \]

Fractional Exhaled Nitric Oxide (FENO)

• 3 isotypes
  – Calcium dependent
    • Endothelial
    • Neurogenic
  – Calcium independent
    • Inducible (main constituent)

• Volatile EBC
  – Measured by its reaction with Ozone by chemiluminescence

• Measured
  – Offline
  – Online
Functions of NO

NITRIC OXIDE

- Increase c GMP
  - Br/vaso dilation
- Deaminates DNA
  - Kills microbe
- Increase Th2
  - IL 4, IL 5, IL 10
- Increase edema
  - Desquamation
Factors affecting FENO

- **Pulmonary**
  - Flow – measured @ constant rate of 50 ml/s
  - Nasal contamination – breathing against closed palate
- **Age**
  - Increases with age esp children
- **Sex**
  - Male > females (recent studies contradictory)
- ** Anthropometric factors**
  - Height - strong +ve corelation
  - BMI & Race - not enough evidence
• Smoking & alcohol
  – Decreases FENO
• Dietary habits
  – Radish, lettuce, water, caffeine & fats – increase FENO
• Medication
  – Steroids & montelukast decreases
  – L-arginine & B agonist increases
• Others
  – Decreases after exercise, bronchoprovocation, spirometry & sputum induction
Reference values

• Difficult to establish due to numerous confounding factors

• Largest study in normal subjects involved > 3,300 pts

• Defined normal value between 24-54 ppb depending on age & height

(CHEST 2007; 131:1852–1856)
FENO in Br asthma

• Over 400 papers looking at various aspects of asthma management
Diagnosis

• Small studies have shown FENO may be an useful screening tool in high risk individuals
• Results from BASALT study evaluating three different strategies in asthma control is to be published later this year
• Atopy
  – Elevated in atopic individuals as a marker of eosinophillic inflammation
  – Atopic asthmatics have even higher levels
  – FENO is also increased in allergic rhinitis
  – Factors need consideration while interpreting FENO values
  – Reduce sensitivity of FENO as a screening tool for asthma in community
• COPD
  – Conflicting data from studies
  – Inversely proportional to FEV1, DLCO, SaO2
  – Normal or only slightly elevated in COPD
    • Smoking decreases NO
    • Possibly due to conversion to peroxynitrite & nitrate
  – Usually elevated during exacerbations
  – Need to evaluate role in certain subsets like Ex-smokers
  – Data emerging for use of CalvNO as marker of early peripheral inflammation
• PAH
  – Etiology is due to reduced vasodilator activity
  – NO is a potent vasodilator in pulmonary circulation
  – Serial FENO levels to monitor disease activity
  – It is inversely proportional to pulmonary artery pressures
  – Increase with successful lowering of pressures with therapy
• ILD
  – Increased due to CalvNO secondary to reduced DLCO
• Lung transplantation
  – For detecting
    • Infection – low sensitivity (57%) - not a good tool
    • BOS – high FENO has a good NPV but low specificity & PPV
    • Acute rejection

• Cystic fibrosis & ciliary dyskinesia
  – Decreased levels
Clinical application

<table>
<thead>
<tr>
<th>Increased FENO</th>
<th>Variable changes in FENO reported</th>
<th>Decreased FENO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma\textsuperscript{1, 79}</td>
<td>Bronchiectasis\textsuperscript{91-93}</td>
<td>Cystic fibrosis\textsuperscript{91, 108-110}</td>
</tr>
<tr>
<td>Late asthmatic response\textsuperscript{80, 81}</td>
<td>COPD\textsuperscript{17, 75, 78, 94-102}</td>
<td>Primary ciliary dyskinesia\textsuperscript{111, 112}</td>
</tr>
<tr>
<td>Allergic rhinitis\textsuperscript{19}</td>
<td>Fibrosing alveolitis\textsuperscript{103}</td>
<td>Pulmonary hypertension\textsuperscript{113}</td>
</tr>
<tr>
<td>Viral infections\textsuperscript{43, 44, 82}</td>
<td>Sarcoidosis\textsuperscript{104}</td>
<td>HIV infection\textsuperscript{114}</td>
</tr>
<tr>
<td>Hepatopulmonary syndrome\textsuperscript{63}</td>
<td>Systemic sclerosis\textsuperscript{105-107}</td>
<td>ARDS\textsuperscript{115}</td>
</tr>
<tr>
<td>Liver cirrhosis\textsuperscript{84, 85}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute/chronic rejection of lung transplant including bronchiolitis obliterans\textsuperscript{86-90}</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


\textsuperscript{1} Thorax 2006;61:817–827.
H₂O₂

• Produced by
  – Superoxide dismutase mediated conversion of superoxide ions

• Detected by
  – Spectrophotometric method using horseradish peroxide

• Overlap in levels found in asthma & COPD hence may be non specific biomarker

• Levels proportional to dyspnoea, sputum neutrophils – s/o disease activity
pH

- Airway acidification & regulation – implicated in pathogenesis of obstructive lung disease
- Unlike other EBCs pH in normal healthy volunteers from different studies similar
- Median pH ~ 8 (data from > 400 subjects)
- This suggest reproducibility across laboratories
- pH is decreased across spectrum of pulmonary diseases in the limited studies available
- Significant overlap across different diseases present
Leukotriene B4

- Produced from arachidonic acid by 5-lipooxygenase
- Estimated using ELISA
- Potent neutrophil chemoattractant – role in airway inflammation
- Mainly been evaluated in COPD & asthma
- Significant variability seen among patients with similar profile across different study groups
- Overlap between pts & healthy controls
8- Isoprostane

- Produced by free radical peroxidation of arachidonic acid
- Supposed marker of oxidative stress in lungs
- Measured by ELISA
- Mainly elevated in COPD & asthma
- Baseline across similar clinical profile is variable in different studies
- Hence repeatability & standardization are difficult to achieve
Prostaglandins

• PGE2 is elevated in stable COPD & asthmatics who are smokers but not in non-smoking asthmatics
• TXB2 is elevated in asthmatics but not in COPD
• Profile of PG may differ in asthma & COPD
• More studies needed to establish normal levels & variability
Other EBC

• Small studies have shown increase as well as positive co-relation with disease activity for various EBC
  – Ammonia
  – Nitrates & nitrites
  – Hydrocarbons (ethane, pentane)
  – CO

• All hampered by size of study, expense, lack of reproducibility, standardization, validation & hence inability for use outside research setting
Serum biomarkers

• Ideal marker
  – Rise before clinical manifestation
  – Easy to measure
  – Help target intervention
  – High sensitivity
  – Consistent results
  – Short half life
  – Cost effective
• Inflammatory biomarkers
  – CRP
  – Procalcitonin
  – S-TREM 1
  – Copeptin
  – Cytokines

• Protein biomarkers
  – CEA
  – CYFRA
  – SP –A & SP - D
CRP

- Acute phase reactant
- Increased in most forms of tissue damage, inflammation & infection
- Liver secretes it in response to IL 6
- Most extensively studied biomarker
- Evaluated in almost all subspecialties of medicine !!!
- Pulmonary diseases
  - COPD
  - Asthma
  - CAP/ VAP & sepsis
CRP in COPD

- Systematic review of studies showed baseline CRP is elevated in stable COPD
CRP in AECOPD

- CRP tends to correlate with severity of exacerbation
- Decreases in responders but data is only from observational studies
- Effect of steroids on CRP unclear
CRP in CAP / VAP

• Prediction of VAP / CAP
  – More so for VAP / HAP in admitted pts
  – Requires serial monitoring (possibly daily)

• Surrogate tool for diagnosis
  – Most sensitive of the available biomarkers for thoracic infections
  – In two studies was better than procalcitonin

• Monitoring therapy
  – Short half life
  – Hence shows decreasing trend in responders
Patterns of CRP course in pneumonia

Pattern A

Pattern B

Pattern C

Pattern D

Current Opinion in Infectious Diseases 2008, 21:157–152
CRP in asthma

• With the advent of hs-CRP several studies have been published recently
• Including one from India
• Salient points
  – Elevated in asthma
  – Co-relate with disease severity
  – May be surrogate marker for systemic inflammation
CRP in sepsis

• It is elevated in sepsis
• Performs better than clinical parameters in predicting infection
• Low sensitivity for differentiating SIRS or non-septic shock from sepsis
• Was hailed as a prognostic marker – same has been challenged in recent trials
• In general inferior to PCT as a biomarker in sepsis
Procalcitonin

In sepsis

• Advantages
  – Relatively specific marker for sepsis
  – Differentiates SIRS from septic shock
  – Absolute & more importantly persistent elevation co – relates with organ dysfunction scores & poor prognosis
  – Serial measurements have more meaning
  – < .5ng/ml & > 2 ng/ml a/w low & high risk respectively for sepsis

• FDA has approved it for use in critically ill with emphasis on
  – Conjunction with other lab & clinical parameters
  – Serial values to be interpreted rather than a one
• Disadvantages
  – Available assays are relatively insensitive for assessment of minor daily variations
  – Though general cut offs have been defined but evidence for same are weak (based on few studies)
  – Marker has been applied over spectrum of diseases, its utility in individual pt needs clinical discretion
  – Utility in presence of renal failure not defined
  – Cost
PCT in pneumonia

• Relatively insensitive for predicting pneumonia in the absence of widespread sepsis

• For deciding whether to start antibiotic
  – Two studies compared procalcitonin based vs. standard protocol for need for antibiotic therapy
  – Significant decrease in duration of therapy & cost with no morality difference
  – Offset by cost of serial procalcitonin
S-TREM 1
In sepsis & pneumonia

• Few single centre trials have shown
  – Sensitive marker for distinguishing sepsis from SIRS
  – Potential as a useful biomarker in sepsis

• Uncertainties
  – No real large RCTs
  – Value of serial measurement unclear
  – Conflicting reports on course of illness & plasma level co-relation
Copeptin

- Secreted along with AVP from pre-pro-vasopressin
- Stable in withdrawn blood for days
- Blood levels have been used in diagnosis of
  - Diabetes insipidus
  - Cardiovascular disease
  - Sepsis
  - Pneumonia
  - AECOPD
- Data for support of its use in pulmonary diseases is emerging
ARDS

• Long PTX 3 was found to be elevated in pts of ARDS in a single trial

• In a recently published study by ARDSnet group
  – IL 8, neutrophil chemotactic factor & SP-D levels were found to be significant in predicting mortality when interpreted with clinical predictors

Chest 2010;137 (2):288-96
Biomarkers in Ca lung

• CEA
  – Elevated in adeno ca & LCLC
  – Limited value when used alone
  – It is used in combination with CYFRA for diagnosis
  – Can also be used to monitor therapy in NSCLC

• CYFRA
  – CYFRA 21-1 potential for monitoring Rx in NSCLC

• Both are non specific biomarker also elevated in other cancers

• ProGRP & NSE are potential tools for diagnosis & monitoring Rx in SCLC

BMB reports 2008; 41(9): 615-625
Summary

• No single biomarker is ideal
• Our understanding of most is still incomplete
• A panel of biomarkers could be more helpful with each supplementing the other
• Need for more reliable assays
• Serial monitoring would hold the key in the future in both acute & chronic pulmonary diseases