Biomarkers in asthma and COPD

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Biomarkers in asthma and COPD

- To provide insight into how disordered cell and molecular processes in these diseases can be used as diagnostic and prognostic tests.
- To offer new knowledge about methods used to find biomarkers related to disease mechanisms.
- To provide some examples of biomarkers that influence the stratification of diseases.
Biomarkers in asthma and COPD

Publications per year

Before 2000: < 100
Biomarkers & asthma
- 2000: 300
- 2010: 600
Biomarkers and COPD
- 2000: 50
- 2010: 250
Biomarkers in asthma

Diamant Pulmonary Pharmacology & Therapeutics 2010

Diagram showing the early and late allergic response, key mediators, and their effects in upper and lower airways.
Biomarkers of COPD: markers of the disease and/or smoking?

- Neutrophil elastase
- Proteinase 3
- Cathepsins
- Matrix metalloproteinases (1, 2, 9, 12)
- Others

Increase:
- α1-Antitrypsin
- Secretory leukoprotease inhibitor
- Elafin
- Tissue inhibitors of matrix metalloproteinases

Decrease:
- Cigarette smoke and other irritants
- Neutrophil chemotactic factors
- Interleukin-8, leukotriene B4
- Neutrophil
- Alveolar macrophage
- MCP-1
- CD8+ lymphocytes

Proteases:
- Neutrophil elastase
- Cathepsins
- Matrix metalloproteinases

Alveolar-wall destruction (emphysema)
- Mucus hypersecretion (chronic bronchitis)
Biomarkers of COPD: markers of the disease and/or smoking?
Why search for biomarkers?

1. Define populations that will derive most benefit from a drug (pharmacogenetics)
2. Improve drug development (pharmacokinetics)
3. Predict disease course (to justify more intense or prolonged treatments) (diagnostics and prognostics)
4. Monitor the effects of therapy (pharmacodynamics)
5. Predict clinical outcomes (surrogate end-points)
6. Monitor adverse events (safety biomarkers)
7. Identify new biological pathways involved in the pathology of COPD and identify new treatment opportunities.

Cazzola M & Novelli G. Pulm Pharmacol Ther 2010
Characteristics of an Ideal Biomarker

- Clear relationship between the biomarker and the pathophysiological events in a disorder - informs on the disease process and prognosis
- Reliable and reproducible in a routine clinical setting
- Inexpensive
- Measurable changes in response to intervention
- Little or no diurnal variation
- Sensitive, disease-specific, high positive and negative predictive values
- Sampling method simple and acceptable to patients

Lesko LJ, Atkinson AJ 2001
Sin and Vestbo 2009
Cazzola M & Novelli 2010
Where to search for biomarkers?

- Blood and urinary samples
- BAL and bronchial biopsies
- Induced sputum
- Exhaled air (gases, EBC, VOCs, ...)

<table>
<thead>
<tr>
<th>Pros and cons of non-invasive lower airways sampling techniques.</th>
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<tbody>
<tr>
<td><strong>Induced sputum</strong></td>
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<tr>
<td>Pros</td>
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<tr>
<td>- Multiple biomarkers</td>
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<tr>
<td>- Reproducible cell differentials on cytopins</td>
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<tr>
<td>- Valid tool for diagnosis (e.g., 'refractory asthma') or</td>
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<td>assessment of anti-inflammatory therapy</td>
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<tr>
<td>Contras</td>
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<tr>
<td>- Representative samples available in approx. 80–90% of</td>
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<tr>
<td>subjects</td>
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<tr>
<td>- Soluble markers subject to dilution</td>
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<tr>
<td>- Non-repeatable over short time-period (&lt;12–18 h)</td>
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<td>- Expertise &amp; experience required (staff/lab)</td>
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<tr>
<td>- Rescue medication needed</td>
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<tr>
<td>- Contraindicated in severe persistent asthma/copd/active</td>
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<tr>
<td>cardiovascular disorders</td>
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<tr>
<td>Overall assessment</td>
</tr>
<tr>
<td>- Validated tool for monitoring of the effects of (novel)</td>
</tr>
<tr>
<td>anti-inflammatory drugs</td>
</tr>
<tr>
<td>- Lengthy, expensive procedure requiring expertise/experience</td>
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<tr>
<td>- Not suitable for patients with severe bronchoconstriction/comorbidities</td>
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Diamant et al. 2010
Examples of biomarkers for asthma

**BAL** - No consistent correlation with asthma severity

Increased numbers of eosinophils - neutrophils in severe asthma

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

**Bronchial biopsies**

- Epithelial shedding, goblet cell hyperplasia, subepithelial collagen deposition
- 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 & IL-5 and eotaxin expression - reduced IL-4 expression of severe asthma
Asthma inflammatory phenotypes

- PAUCYGRANOCYTOPENIC
- NEUTROPHILIC
- May-Giemsa staining
- Neutrophil elastase stain
- Haematoxylin and eosin stain

Allergens
Sensitizing agents
Steroid reduction
Others

Viral & bacterial infections
Cigarette Smoking
Pollutants
Athlete /obese
Occupational
Others

CS-resistant asthma ?
High-doses of CS ?
Others
How can a biomarker might be used to reduce future risk in asthma

1) it might be used to improve overall asthma control by optimising maintenance anti-inflammatory therapy.
   - AHR (methacholine) and induced sputum eosinophil counts have been used to adjust ICS dose

2) a biomarker might be used to predict individual asthma events
   - sputum eosinophil counts to predict future loss of control?

Taylor 2009
Exhaled nitric oxide (FENO)

May have a role in asthma for:

- The diagnosis of airways disease
- (severe asthma)
- The identification of corticosteroid responsive disease
- To guide the titration of corticosteroid dose
- Follow-up and risk analysis?
Suggested algorithm for assessment of patients presenting with untreated airway disease

Pavord et al
J Asthma 2008
Exhaled nitric oxide (FeNO)

On changes in sputum eosinophils and FeNO after reducing ICS therapy

“The changes in sputum eosinophils correlated with subsequent negative changes in symptoms and lung function, and were a significant predictor for reduction in ACQ score (ie, improved asthma control)”

Jatakanon 2009
Evaluation of asthma control with induced sputum eosinophilia


Does a strategy that minimized airway eosinophilia reduces asthma exacerbations compared to a standard management strategy?

- 74 patients with moderate to severe asthma
- BTS Guidelines vs eosinophils control
- Assessment over a 12 months period

<table>
<thead>
<tr>
<th>Severe exacerbations</th>
<th>Hospital admissions</th>
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<tr>
<td>BTS</td>
<td>109</td>
</tr>
<tr>
<td>Sputum eosinophils</td>
<td>35</td>
</tr>
</tbody>
</table>

(p = 0.01)

(p = 0.047)
LOMA Study

- Eosinophilic: 38.7% (P = 0.008)
- Non eos/non neu: 62.5% (P = 0.009)
- Neutrophilic: 21.0% (P = 0.2)

Clinical Strategy vs Sputum Strategy
Asthma exacerbations and induced sputum analysis

Biomarkers in **BAL (COPD)**

- The predominant (80%) 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.
- Eosinophil cationic protein (ECP), myeloperoxidase, and IL-8 are frequently increased in patients with COPD and in healthy smokers.
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<th>Biomarkers in <em>bronchial biopsies (COPD)</em></th>
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<tr>
<td>- Increase in macrophages and CD8$^+$ T-cells; eosinophils are increased - lower counts in COPD patients with chronic bronchitis</td>
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<td>- Increased expression of CCL5 and CXCL7 in the bronchial mucosa if stable</td>
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<tr>
<td>- The number of IL-22$^+$ and IL-23$^+$ immunoreactive cells is increased in the bronchial epithelium of stable COPD compared with control groups (IL-17A$^+$ and IL-22$^+$ immunoreactive cells increased in the bronchial submucosa of COPD compared with control non-smokers)</td>
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<tr>
<td>- The number of cells expressing YKL-40, a chitin-binding protein that is elevated in patients with various inflammatory conditions.</td>
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<tr>
<td>- Altered surfactant protein (SP)-A expression in human lung tissue samples obtained from patients with COPD</td>
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</table>
Induced sputum: COPD

- Increase in the percentage of neutrophils
- Inflammatory mediators involved in neutrophil recruitment & metabolism (e.g. IL-8, GROα, LTB₄, neutrophil elastase, MCP-1, and human neutrophil lipocalin [HNL]) + MPO elevated in sputum from stable COPD patients
- Increased MMPs-1, -8, -9 and TIMP-1
Exhaled biomarkers: COPD

- $\text{FE}_{\text{NO}}$ does not seem to be useful, as the levels are usually normal or only slightly elevated, except during exacerbations (due to increase in oxidative stress, resulting in formation of peroxynitrite and then nitrate, so that NO is removed from the gaseous phase)

- An increase in $\text{FE}_{\text{NO}}$ in patients with COPD is correlated with increased numbers of eosinophils, an increased bronchodilator response, and steroid responsiveness, and so may be useful in detecting associated asthma, but it has no clear prognostic utility

- Using a two-compartment model as a research tool it has been demonstrated that NO is elevated in the alveolar compartment of COPD patients and correlated with disease severity
Blood markers in COPD

- Surfactant protein D (SP-D)
- Fibrinogen
- CRP
A review of 652 studies suggested that few biomarkers have been validated, and there is little information about reproducibility and the relationship to disease development, severity, or progression.

Only sputum neutrophils and interleukin (IL)-8, as well as serum tumour necrosis factor (TNF)-α and C-reactive protein (CRP), showed any trend toward separating different stages of COPD.

Franciosi LG et al. Pulm Pharmacol Ther 2006
Procalcitonin and C reactive protein in hospitalised adult patients with community acquired pneumonia, exacerbation of asthma and COPD

Bafadhel et al. CHEST 2010

Procalcitonin and CRP levels helped distinguish pneumonia from exacerbations of asthma. CRP levels could be used to guide antibiotic therapy.
Some « sophisticated » methods to find biomarkers ...
Criteria for developing novel biomarkers

The important factors in relation to biomarkers and disease characteristics or drug response in either the scientific advice or regulatory approval process include:

1. The identification of whole-OMIC high throughput platforms (genomics, transcriptomic, proteomics, metabolomics, etc.) for biomarkers identification and validation;

2. The evaluation of customized/gene specific assay for biomarkers validation in patients’ cells/tissues/samples and the evaluation of bioinformatics tools.
Biomarkers of COPD: Genetic markers

immunological response (3261, 52%)
- cytokines and chemokines
- major histocompatibility complex
- oxidative stress
- receptors
- others

remodeling (1261, 20%)
- growth factors and cell differentiation
- proteolytic enzymes
- receptors
- proteolytic enzymes
- structural proteins
- transcription factors
- others
Biomarkers of COPD: Gene expression

- 220 genes whose expression in lung tissue was associated with COPD-related phenotypes

- The ability to identify COPD-related processes in airway gene expression raises the possibility of developing airway biomarkers that could be used clinically to identify smokers at higher risk for developing disease as well as to serve as intermediate biomarkers of efficacy for novel and existing COPD therapies.
Electronic Nose (olfactometry)

- Non-invasive and portable - Uses high-dimensional biomarker signal - Produces individual signature: « breath » print
- Potential tool for diagnosis and monitoring of anti-inflammatory therapy
- Technology still developing & validation procedures to perform - Complex analyses
- Promising technique for both clinical and research applications
- Could differentiate asthma from COPD from Normals (P Sterk’s group)
Proteome analysis to find new biomarkers in EBC for asthma diagnosis and follow-up

- The use of proteome analysis may reveal disease-specific proteolytic peptide or protein patterns, and help find novel proteins for the detection of asthma.

- Liquid chromatography and mass spectrometry used to separate and detect proteins (proteolytic peptides) present in EBC samples from 30 healthy children and 40 children with asthma in the age group of 6-12 years.

- Support vector machine analysis resulted in differentiating profiles based on asthma status but no correlation with spirometry, FENO, and EBC pH or LTB4.

- The more abundant proteins in EBC were identified as cytokeratins, albumin, actin, haemoglobin, lysozyme, dermcidin, and calgranulin B.

- This study shows that EBC contains proteins that are of interest for future non-invasive.

Figure 1. Example urine NMR spectrum showing the significant bins in an expansion of the aromatic region. The trigonelline peaks correspond to the significant bin at 8.81-8.87 ppm (see Table 4). The tall hippurate peaks (on right) correspond to the significant bins at 7.82-7.88, 7.62-7.66 and 7.51-7.57 ppm. The broad hippurate peak (on left) corresponds to the 8.50-8.56 and 8.56-8.61 ppm bins. Formate was typically lower in concentration than the other two compounds and corresponds to the significant bin at 8.43-8.48 ppm. Output from Chenomx Profiler 5.0.
Combined markers?

Receiver operating characteristics (ROC) curves for FeNO, EBC pH and their combination in the identification of well-controlled asthma

Kotsikas et al. Resp Med 2010
Conclusions

- There is an intense research effort on biomarkers in respiratory diseases
- Can help in the diagnosis and prediction of outcomes – can guide therapy
- Promising but the relationships with disease activity and outcome to better define
- Search for the ideal markers...