Inflammatory biomarkers in severe asthma
Stephen T Holgate,
IIR Division,
School of Medicine,
University of Southampton

sth@soton.ac.uk

Asthma – more than inflammation - the airways in asthma undergo remodelling

Asthma is a complex disease

Heterogeneous disease requires multiple biomarkers for accurate diagnosis
Wadsworth A et al. J Asthma Allergy 2011; 4: 77-86

Pros and cons of some currently used asthma biomarkers
Adapted from Wadsworth A et al. J Asthma Allergy 2011; 4: 77-86

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary function e.g. spirometry, PEF, BHR</td>
<td>Non-invasive, sensitive, well validated</td>
<td>Unable to detect sub-phenotypes, reflect disease mechanisms or predict treatment responses</td>
</tr>
<tr>
<td>Tissue biopsy</td>
<td>Definitive measure of airway pathology</td>
<td>Highly invasive, require expertise</td>
</tr>
<tr>
<td>Induced sputum</td>
<td>Less invasive, reflects airway inflammation, useful to monitor CS treatment</td>
<td>Somewhat uncomfortable, requires expertise, reproducibility variable, difficult in children</td>
</tr>
<tr>
<td>Exhaled NO (eNO)</td>
<td>Non-invasive, simple measurement technique, Highly sensitive to CS</td>
<td>A subset of asthma, care to standardise</td>
</tr>
</tbody>
</table>

Links between pathologic mechanisms and clinical consequences in asthma.

Sputum and circulating eosinophils are accepted as biomarkers of asthma, but lack discrimination for subphenotyping beyond “Th2 high” and “Th2 low”

Sputum and circulating eosinophils are accepted as biomarkers of asthma, but lack discrimination for subphenotyping beyond “Th2 high” and “Th2 low”

Sputum eosinophilia as a reliable biomarker of subtypes of severe asthma

Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. Green RH. et al Lancet. 2002; 360: 1715-21

Use of exhaled nitric oxide to identify a reactive, at-risk phenotype among patients with asthma
Dweik RA et al. Am J Respir Crit Care Med. 2010; 181: 1033-41

FeNO and airway inflammation, airflow limitation, hyperinflation, BHR and atopy determined in 446 asthmatics (175 severe, 271 non-severe) and 49 healthy subjects from NIH SARP.

- All patients with asthma and high FeNO had more BHR, allergic airway inflammation (sputum eosinophils), atopy (positive skin tests, higher serum IgE and blood eosinophils), and hyperinflation, but decreased awareness of their symptoms.
- High FeNO identified those patients with severe asthma characterized by the greatest airflow obstruction and hyperinflation and most frequent use of emergency care.

How about airway biopsy?

- High FeNO identified those patients with severe asthma characterized by the greatest airflow obstruction and hyperinflation and most frequent use of emergency care.

Mast cell phenotype, location, and activation in severe asthma. Data from the Severe Asthma Research Program (SARP)

(A) Distribution of submucosal total mast cells (MCs) (tryptase-positive MC\textsubscript{Tot}) and chymase-positive MCs (MC\textsubscript{Ch}) by immunostaining. Open circles in the severe asthma group represent subjects who are not on systemic corticosteroid.

While good at identifying subphenotypes biopsy is not practical.

Are there urinary or circulating biomarkers of airway inflammation?

High levels of urinary leukotriene E\textsubscript{4} excretion in steroid treated patients with severe asthma

Urinary leukotriene E\textsubscript{4} (LTE\textsubscript{4}) in mild–moderate asthmatics (n=25), severe asthmatics (n=40) and control subjects (n=20).

Eicosanoids in Exercise-induced asthma

Insufficiently discriminant

Sensitivity of biomarkers for eosinophilic airway inflammation

A, Probability of composite eosinophil status = "high" as a function of serum periostin. Dashed lines denote 95% CIs.

As asthma severity increases neutrophils become part of the inflammatory picture

Differences in airway cytokine profile in severe asthma compared to moderate asthma: marked increase in epithelial an smooth muscle CXCL8 (IL-8)

Interferon γ

CXCL8 Moderate asthma

CXCL8 Severe asthma

Smooth muscle
Immunostaining of epithelium for IL-8 and number of cells per field in subepithelium of bronchial biopsy samples staining for IL-8 (CXCL8) in moderate & severe asthma


An ex vivo model of severe asthma using reconstituted human bronchial epithelium

- Human bronchial epithelial cells derived from bronchial biopsy specimens in mild and severe asthma cultured for 21 days in an air-liquid interface to form a fully differentiated airway epithelium.

Intrinsic abnormality in the epithelium to adopt a chronic wound and pro-inflammatory phenotype
- Greater levels of mucin secretion
- Released more CXCL8
- Produced lower levels of lipoxin A(4)
- Higher expression of gene for 15-lipoxygenase 2

Application of metabolomics in asthma is becoming a reality


Morphologic analysis of bronchial epithelia in air-liquid interface cultures

Some alternative sources of biomarkers
Adapted from Wadsworth A et al. J Asthma Allergy 2011; 4: 77-86

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exhaled breath condensate &amp; exhaled volatiles</td>
<td>Non-invasive, multiple biomarkers to enable subphenotyping</td>
<td>Highly variable, limited to small MW analytes, salivary contamination (EB)</td>
</tr>
<tr>
<td>Serum or plasma proteins</td>
<td>Less invasive, multiple biomarkers, standardised operating procedures established</td>
<td>Less airway specific, reflects subtle changes within circulating compartment</td>
</tr>
<tr>
<td>Urinary metabolites (&gt;70)</td>
<td>Non-invasive, multiple biomarkers, SOPs established, good sensitivity but variable specificity</td>
<td>Unproven clinical use, limited access to analytical equipment e.g. NMR, MS</td>
</tr>
</tbody>
</table>
Immunological biomarkers in sera correlated with asthma control and quality of life measurements from chronic asthmatic patients.


1. Sera from moderate and severe persistent asthma and normal controls
2. 50 analytes, including cytokines, chemokines, angiogenic, and growth factors determined by multiplex assay.
   - 12 of 29 cytokines higher in patients with asthma than controls, but only IFNγ significantly lower in asthma than controls. (IL)-3 and IL-18 levels were significantly higher in poorly controlled disease.
   - 5 of 12 chemokines higher in patients with asthma than controls.
   - 5 of 6 growth factors higher in patients with asthma than controls, and 3 were higher in those poorly controlled.
3. IL-18, FGF2, HGF, and SCF correlated with poor asthma control and reduced quality of life

The application of proteomics


Proteomic pathway analysis to define different asthma endotypes: “molecular taxonomy”


Asthma sputum interactome  Functional analysis of the sputum proteome

Creation of a New Taxonomy requires an “Information Commons” in which data on large populations of patients become broadly available for research use and a “Knowledge Network” that adds value to these data by highlighting their inter-connectedness and integrating them with evolving knowledge of fundamental biological processes.

Towards personalised medicine: reclassification of human disease by identifiable causal pathways

The key to the success of stratified or personalised medicine will be the integration of complex data sets from multiple sources and the development of multidisciplinary research