Pulmonary Biomarkers in Chronic Obstructive Pulmonary Disease

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Chronic Obstructive Pulmonary Disease is characterized by airflow limitation that is not fully reversible, chronic lung inflammation and extra –pulmonary systemic effects. It includes emphysema, chronic bronchitis and disease of the small airways.

The progressive and non-reversible airflow limitation has been evaluated by spirometry for many years.Current disease staging systems are mainly based on FEV1- a spirometric measure.

Aiming to unravel the various mechanisms causing chronic inflammation and thereby offering appropriate therapies for COPD has led to the evaluation of biomarkers.

A BIOMARKER is defined as a biological marker that is objectively measured and evaluated as an indicator of normal biological process, pathogenic process or pharmacologic response to a therapeutic intervention. Biomarkers can be genetic polymorphisms or parameters that may be used for assessment of disease severity or effects of therapy.

Sources of biomarkers:

- Serum
- Exhaled gases & breath condensate
- Induced sputum
- Bronchoalveolar lavage fluid
- Bronchial biopsy

Serum Biomarkers:

Serum has been tested for 36 biomarkers till date. They include C-reactive protein, fibrinogen, macrophages, neutrophils,eosinophils, surfactant protein D, I L 6,I L 8, ANP,BNP,TNF α, neopterin, procalcitonin, adrenomedullin etc.

J.Hurst et al reported a RCT wherein 15 systemic proteins like BD2 (β-defensin-2), CC16,CRP,IL-6,IL-8,IP-10,MMP-9,MPO and others were assessed in 42 patients.They found a significant response in IL-6 levels.

Another study-ECLIPSE(evaluation of COPD longitudinally to identify predictive surrogate endpoints) by Miller et al assessed the profile of 36 blood biomarkers in 275 COPD subjects to identify potential diagnostic and surrogate biomarkers.CCL-18,BD-2,MMP8,MMP9,MPO,SP-D(surfactant protein-D),CC-16 and fibrinogen have emerged as promising biomarkers.

Advantage:

- Blood can be easily obtained.
- Measuring equipments are reasonably cost-effective.

Disadvantage:

- Blood biomarkers can be modulated by morbidities other than COPD.
- They may represent epiphenomena unrelated to COPD.

A wide range of pulmonary and serum biomarkers, several kinds of clinical samples and different methods of detection are available, each with its range of sensitivity. The clinical scenario-stable disease or exacerbation stage of COPD also matter.The rate and magnitude of change in the biomarker also matters. The routine use of biomarkers in the management of COPD depends on the resources available; the biomarker concentration should be measured within the first hours of admission, preferably before starting treatment; serial measurements during hospital stay and follow-up levels may be required; this limits the use of biomarkers to hospital premises only. Easy and reliable assays

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are required for different biomarkers that can be used by both family physicians i.e. at home/OPD and by hospital specialists.

The questions, which biomarker and when ,and their standard values remain unanswered. Further trials are required to determine the biomarkers to be tested in stable COPD and in exacerbations; the usefulness of inflammatory markers in distinguishing exacerbations of infectious origin from non infectious causes will ensure a more rational therapeutic approach-antibiotherapy or immunomodulatory treatment.

The correlation of certain biomarker levels with prognostic markers of COPD progression might help us to identify patients with poorer prognosis and higher risk of treatment failure.

Exhaled Gases & Breath Condensate

Nitric oxide, carbon monoxide& hydrocarbons such as ethane and pentane have been detected in exhaled gases. Exhaled nitric oxide correlates with airway inflammation in bronchial asthma; it is however less useful in COPD. Exhaled carbonmonoxide is elevated in patients with COPD and also in normal smokers and sustained ex-smokers. Its levels are confounded by environmental CO levels and smoking. Ethane levels are elevated in COPD and correlate with disease severity; but measuring the levels by gas-chromatography –mass spectrometry is difficult.

pH of exhaled breath condensate, levels of hydrogen peroxide, 8-isoprostane, malondialdehyde and inflammatory mediators like leukotriene B4, prostaglandin E2, IL-6 Tumour Necrosis Factor - A in exhaled breath condensate have been evaluated in stable disease and exacerbation. Hydrogen peroxide, 8 –isoprostane and malondialdehyde levels are increased in stable state and further rise in exacerbations. pH of breath condensate is decreased in COPD.

Increased concentration of inflammatory mediators like leukotriene B4, IL-6, Tumour Necrosis Factor - α and PGE 2 have been reported in COPD patients.

However, extensive variable dilution due to water vapour during condensation and low concentration of the biomarkers are the major limitations .

Advantage

- This is non-invasive
- Repeated sampling is easy.

Disadvantage

- Variability of measurement
- Low concentration of mediators.

Induced Sputum

Spontaneously produced sputum normally observed in many COPD patients may contain a high proportion of dead cells; this may result in misleading cell counts and mediator measurement. Hence induced sputum is chosen.

Sputum is assessed for cells and inflammatory mediators

Cells -1)Increase in total number of neutrophils and macrophages and in some patients eosinophils(indicating concomitant asthma /response to glucocorticoids)

2) Increase in CD 8 T cells has also been demonstrated.

The two main cell types in sputum-neutrophils and macrophages-showed a significant difference between healthy state and each of the stages of COPD, while eosinophils did not. Sputum neutrophil studies demonstrate an upward trend from healthy to severe stages of COPD.Significant reduction in neutrophils is reported with low dose oral theophylline; but, no change with inhaled or oral glucocorticoids.

- Inflammatory mediators: IL-8 is increased in COPD and is related to severity: it rises further in exacerbations.TNF-α, IL-6, Leptin, Fibrinogen have been detected in higher concentrations in patients of COPD.Increased concentrations of proteases –neutrophil elastase, matrix metalloproteinase -8 (MMP-8), MMP-9, MMP-12, Interferon inducible protein -10 have been noted in sputum; MMP-9 more so in patients of emphysema: hyalurone is found to be higher in smokers with COPD.

Studies involving sputum fibrinogen, macrophages, C-reactive protein have also been

done: results are inconsistent.

Advantages

- Easy to obtain.
- Adequate and satisfactory yield of inflammatory cells and mediators.

Limitations

- Obtained predominantly from proximal large airways and may not reflect peripheral inflammation.
- Induction with hypertonic saline promotes neutrophilic inflammation that persists for 24 hours and thus repeated sampling in this period is not possible.
- Solubilization of sputum with dithiothreitol (DTT) disrupts sulphydryl bonds and may alter proteins and cytokines.
- Proteases in sputum may degrade certain protein mediators.

Bronchoalveolar Lavage (BAL)

The BAL fluid is assessed for cells and inflammatory mediators.

- Cells –Alveolar macrophages predominate in COPD .Neutrophils, T –lymphocytes and eosinophils are also present.
- The macrophages show reduced activity of histone de acetylase -2(HDAC-2) which causes activation of transcription factor nuclear factor-kB (NF–kB) in bronchial epithelial cells.

Inflammatory mediators :

- Eosinophilic cationic proteins, myeloperoxidase, IL-8 are increased in COPD patients: these are also raised in healthy smokers.
- Proteases and anti-proteases are also detectable: increase in elastase and decrease in anti-elastase activity is noted in COPD patients.

Three studies so far have assessed the effects of treatment on cellular and mediator components of BAL. Though the numbers are small, they suggest inhaled glucocorticoid treatment is beneficial.

Advantage: Lung periphery can be sampled.

Disadvantage

- Invasive procedure.
- May cause transient fever
- Fluid recovery is limited in patients of emphysema.
- Dilutional effect of saline lavage causes difficulty in quantification of biomarkers.

Bronchial Biopsy

Though the lung parenchyma and small airways are predominantly involved in COPD, bronchial biopsy may give insight into the pathogenesis.

Studies involving bronchial biopsy usually consist of

1) A baseline biopsy and then a second biopsy after a defined period of treatment OR

2) A single biopsy at the end of treatment and a biopsy in a parallel group taking placebo.

Bronchial biopsies are assessed for a) cells and b) inflammatory mediators.

a) Cells: In patients of stable COPD, there is increased infiltration of macrophages and activated T-lymphocytes. Exacerbations are characterized by increased number of eosinophils and neutrophils; these cause upregulation of chemokine receptors-CCL5 and CXCL5.

b) Mediators: The activity of HDAC2 and NF-kB in the bronchial epithelial cells has been studied.

CRP, I L-6, I L-8, TNF-α, Fibrinogen have also been studied.

Advantage:

Direct sampling of airway tissue maintaining spatial relationships of structural components such as epithelium, basement membrane, vessels,connective tissue, smooth muscle and submucosal glands

- Invasive
- Proximal airways are studied and not distal airways .
- Cannot be performed on patients with comorbid conditions
- Since effect of treatment is assessed, larger

number of subjects is required and submucosal glands

A meta-analysis of cell types in bronchoalveolar lavage bronchial biopsies is not possible due to limited number of studies and data. The overall conclusion is that greater anti-inflammatory effects are obtained with PDE-4 inhibitors or glucocorticoids with longacting β2 agonists, but they are not related to functional or clinical improvements. Inhaled glucocorticoids seem to have a little effect on the airway inflammation.

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