

## ***Online supplement 1.***

### **EBC mediators in different diseases**

#### ***pH***

EBC pH has been investigated in variety of diseases [1]. De-aerated EBC pH is lower in acute asthma and normalises with corticosteroid treatment [2], whereas it is not lower in stable treated patients with severe and non-severe asthma in the Severe Asthma Research Program [3]. COPD patients present lower deaerated pH values than non-smokers without COPD [4]. EBC pH has been related to hyperinflation and air trapping in ex-smokers with COPD [5]. In longitudinal follow-up study, using EBC pH at standardised pCO<sub>2</sub> level, small changes could be followed in patients with COPD during exacerbations [6]. When the pH is determined by the CO<sub>2</sub> gas standardization method [7], no acidification in patients with COPD [8], CF [9], lung cancer [10] or bronchiolitis obliterans syndrome [11] can be detected. In contrast, exacerbation of asthma is associated with lower pH in the airways [6]. Between-day variability of EBC pH is similar among different groups of pulmonary patients [5-12].

#### ***Hydrogen peroxide and nitrogen oxides***

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is a volatile marker of oxidative stress in EBC [1, 13]. H<sub>2</sub>O<sub>2</sub> in EBC is best measured during or immediately after collection, because its concentration decreases quickly and unpredictably in the sample [1, 14]. EBC H<sub>2</sub>O<sub>2</sub> is elevated in asthmatic patients; the meta-analysis of Teng et al. suggests a relationship between EBC H<sub>2</sub>O<sub>2</sub> and asthma severity and control [15]. EBC H<sub>2</sub>O<sub>2</sub> levels are also elevated in COPD patients and correlate with disease severity [16].

#### ***Nitric oxide related compounds in EBC***

Exhaled NO assays measure the quantity of NO that emanates from the airway and not the amount of NO that is formed. Consumptive processes – including oxidation reactions – may decrease the amount of gas phase NO available for exhalation. Higher oxides of nitrogen are resulting reaction products, and are easily measured in EBC.

Assessing all nitrogen oxides in exhaled breath provides more detailed interpretation of exhaled NO [17]. Furthermore, Carraro et al. showed that asymmetric dimethylarginine

(ADMA), which acts as endogenous inhibitor of NOS, was elevated in EBC of asthmatics supporting a role for ADMA in asthma pathogenesis [18].

#### ***Eicosanoids, isoprostanes, adenosine, ATP, other purines and lipid peroxidation products***

EBC eicosanoids and F2-isoprostanes represent a non-invasive tool for exploring the pathophysiology of respiratory diseases. Using immunoassays, leukotrienes (LTs), prostanoids and 8-isoprostane are increased in EBC of patients with asthma, COPD and cystic fibrosis. Liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-MS enable an accurate and precise assessment of LTB<sub>4</sub> [19], LTC<sub>4</sub>, LTD<sub>4</sub> and LTE<sub>4</sub> [20], 8-isoprostane [21], and prostaglandin E<sub>2</sub> [21] concentrations in EBC in different airway diseases [22]. Some studies reported the potential utility of EBC eicosanoid and F2-isoprostane for assessing the effects of pharmacological treatment in patients with respiratory disease [1, 22-24].

Adenosine, ATP and other purines have been detected in EBC of patients with different respiratory disorders including asthma, COPD and CF by different methodology and was used to assess short-term changes in purinergic signalling of the airways during exercise-induced bronchospasm and also suggested to be used for tracking longitudinal changes in cystic fibrosis [1, 25, 26].

Finally, by-products of polyunsaturated fatty acid peroxidation, for example, malondialdehyde (MDA) can also be detected in EBC of patients with asthma, COPD and CF using HPLC [27,28]. Although elevated MDA levels reflect increased oxidative stress in these conditions, a recent study indicated that the clinical applicability of the measurement may be limited, possibly due to the high day-to-day variability for this marker in EBC [29].

#### **Metabolomics and proteomics**

Analysis of metabolomics in EBC with high-resolution nuclear magnetic resonance spectroscopy or mass spectrometry (MS) techniques enables a specific quantitative description of the endogenous metabolites, providing a metabolic "fingerprint" which can be used for classification purposes. This approach can be used for unbiased biomarker

discovery and unravelling the metabolic changes that characterise respiratory diseases. EBC metabolomic analysis can distinguish between healthy subjects and patients with various respiratory diseases, including COPD [30], stable and unstable CF [31], asthma of different inflammatory phenotypes [32], and severity [33] and primary ciliary dyskinesia [34].

Profiling the proteome of EBC is another challenging approach to study airway diseases and phenotype pulmonary patients. Application of protein microarray to EBC allows the simultaneous detection of many potential biomarkers, and thus presents an excellent tool for proteomic analysis of EBC in patients with COPD or asthma [35,36]. In some studies pooled samples are used in order to increase the protein concentration of EBC. Using this technique, it has been demonstrated that the EBC cytokine pattern of patients with certain pulmonary diseases (lung cancer, bronchiolitis obliterans) is clearly different from that of healthy subjects [37,38]. These data suggest that proteomic analysis of EBC is a useful approach to gain information about molecular signatures linked specifically to pulmonary diseases.

*Recommendations-Future research:* Further clinical studies are needed in EBC including the establishment of reference values.

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