CHEST

Original Research

COPD

Exhaled Metallic Elements and Serum Pneumoproteins in Asymptomatic Smokers and Patients With COPD or Asthma*

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Study objectives: **The aim of this study was to characterize the elemental composition of exhaled breath condensate (EBC) in order to identify new biomarkers of exposure and susceptibility in COPD patients. Serum pneumoproteins were used as lung-specific biomarkers of effect.**

Design: **EBC was obtained from 50 healthy subjects, 30 healthy smokers, 30 asthmatics, and 50 patients with stable COPD, and was collected by cooling exhaled air. Trace elements and toxic metals in the samples were measured by means of inductively coupled plasma-mass spectrometry and electrothermal atomic absorption spectroscopy. The serum pneumoproteins were immunoassayed.**

Results: **The EBC of COPD subjects had higher levels of such toxic elements as lead, cadmium, and aluminum, and lower levels of iron and copper, than that of the nonsmoking control subjects. There were no between-group differences in surfactant protein (SP)-A and SP-B levels. Clara-cell protein and SP-D levels were negatively and positively influenced, respectively, by tobacco smoke.**

Conclusions: **Our results show that toxic metals and transition elements are detectable in the EBC of studied subjects. We propose new biomarkers of exposure as a means of assessing the target tissue dose of carcinogenic and pneumotoxic substances from tobacco smoke or polluted workplaces, and the use of the transition elements involved in redox systems of oxidative stress as disease biomarkers associated with effect or susceptibility. Together with biomarkers of effect, such as serum pneumoproteins, the elemental composition of EBC may be clinically useful in distinguishing similar diseases.** ***(CHEST 2006; 129:1288–1297)***

Key words: COPD; exhaled breath condensate; metals; pneumoproteins; trace elements

Abbreviations: ANOVA = analysis of variance; CC16 = Clara-cell secretary protein; EBC = exhaled breath condensate; ETAAS = electrothermal atomic absorption spectroscopy; ICP-MS = inductively coupled plasma-mass spectrometry; LOD = limit of detection; PM = particulate matter; SOD = superoxide dismutase; SP = surfactant protein

COPD is one of the leading causes of morbidity and mortality worldwide, and represents a substantial economic burden on global health.[1] It is characterized by small airways disease, mucus hypersecretion, and chronic bronchitis, which lead to an obstructed airflow that impairs ventilatory capacity and gas exchange, and causes shortness of breath.[1]

Various instruments have been developed for diagnosing, monitoring, and evaluating COPD. Lung function tests offer insights into changes in airway caliber, exhaled air flow, lung volumes, and gas exchange,[2] and imaging techniques (especially high-resolution CT) offer insight into the loss of lung tissue[3]; both provide indirect measurements of the adverse effects occurring in a biological system as a consequence of exposure to toxic or noxious agents. More direct evaluation of biological lung events can be obtained using more or less invasive measurements (bronchoscopy, induced sputum). These have improved our understanding of the biological processes occurring in lung diseases and still represent the "gold standard" for evaluating the diseases themselves,[4] but their applicability is limited mainly because the invasiveness of the sampling procedures makes them unsuitable for routine clinical use.

Modern research relies on biomarkers, which are

defined as any substance, structure, or process that can be measured in the body or its products, and which influence or predict the incidence of outcome or disease.[5] Biomarkers of lung diseases can be developed on the basis of exhaled air and blood analyses.

The analysis of exhaled air is feasible and noninvasive.[6] Exhaled breath condensate (EBC) obtained by cooling exhaled air under conditions of spontaneous breathing is a promising biological fluid that could provide a real-time assessment of pulmonary pathobiology. It can be easily and noninvasively collected from patients of any age using portable devices in an outpatient setting or even at home. EBC is particularly suitable for the sequential and longitudinal sampling of the lower respiratory tract, and published data on inflammation mediators suggest that it reflects the abnormalities noted in bronchoscopic specimens.[7] Despite the enthusiasm of a few research groups, there is skepticism concerning the diagnostic and monitoring validity of EBC because of the analytical problems associated with measuring trace amounts of unstable and nonspecific mediators, which mainly relies on immunochemistry techniques that lack reference methods and materials, and are affected by their poor sensitivity, specificity, and selectivity.[8]

As EBC mainly consists of water that is practically free of potentially interfering solutes, it is an ideal biological fluid for elemental determinations based on relatively common techniques, such as electrothermal atomic absorption spectroscopy (ETAAS), or less frequently available reference instruments such as inductively coupled plasma-mass spectrometry (ICP-MS).

The aim of this study was to investigate the elemental composition of EBC in order to identify new biomarkers of exposure, or susceptibility, in COPD patients. The working hypothesis was that long-term exposure to tobacco smoke (which may cause the development of COPD) leads to an increased lung uptake of toxic metals that, because of their stability, can also be used as tracers of environmental pollution. Likewise, hard metals in occupationally exposed workers[9] and toxic metals (such as lead, cadmium, chromium, nickel, and aluminum) should provide a quantitative estimate of target tissue burden.

EBC was also used to quantify essential elements as biomarkers of susceptibility, which have been defined as "indicators of an inherited or acquired limitation of an organism's ability to respond to the challenge of exposure to a xenobiotic substance."[5] Individual detoxifying capacity modulates the lung response to inhaled pneumotoxic substances, and there is considerable variability in individual responses to toxic substances. A growing body of evidence indicates that many transition elements play important roles in biological processes by activating or inhibiting enzymatic reactions, by competing with other elements and metalloproteins for binding sites, or by affecting the permeability of cell membranes.

The possible biomarkers of susceptibility were investigated bearing in mind that some transition elements play a fundamental role in the respiratory chain (iron), or are components of mitochondrial (manganese) or cytoplasmic (copper, zinc) superoxide dismutases (SODs), or glutathione peroxidase (selenium). Given their key roles in the generation and detoxification of reactive oxygen species, and the scavenging of free radicals, transition elements modulate the response to toxic substances,[10] thus possibly accounting for the limited proportion (15 to 20%) of smokers acquiring COPD. Biomarkers of susceptibility may be useful in identifying and counseling people at increased risk of disease when exposed to tobacco smoke or environmental pollutants.

Finally, we analyzed serum pneumoproteins (Clara-cell secretory protein [CC16]), and three surfactant proteins (SPs), SP-A, SP-B, and SP-D, which were used as a complementary approach to develop biomarkers of effect suitable for the long-term monitoring of COPD patients. As these proteins are mainly (if not exclusively) secreted within the respiratory tract, their occurrence in the vascular compartment can only be explained by assuming their leakage from the lung into the bloodstream. Excluding changes in renal function, their serum concentrations can be expected to reflect their rate of synthesis and the permeability of the lung epithelium.[11]

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