Reproducibility of exhaled biomarkers in COPD—the road less traveled

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In recent years, interest in the measurement of exhaled biomarkers has increased, mainly driven by the unmet clinical need to monitor airway inflammation and the response to antiinflammatory therapy. Beside exhaled nitric oxide measurement that entered clinical practice, measurement of biomarkers in exhaled breath condensate (EBC) is a rapidly expanding area of this field. EBC is easy to collect as it only requires the noninvasive collection of exhaled breath in a cold trap. The obtained fluid is a complex diluted solution of diverse biomarkers with various chemical stabilities. Due to the complexity of EBC and the fact that it is a much diluted sample, there are still several unsolved issues about standard protocols for measurements and there is a lack of reproducibility data for different biomarkers, particularly in disease states. The lack of knowledge and evidence on certain areas limited the ability of the European Respiratory Society/American Thoracic Society Task Force to make firm guidelines for each aspect of this sampling technique and prompted the authors to highlight areas for further research (Horvath et al 2005).

The current issue of the *International Journal of COPD* contains an important methodological contribution to this rapidly growing field (Borrill et al 2007). The authors performed a carefully designed study to assess within assay, within and between day reproducibility of EBC leukotriene B_4 (LTB_4) and 8-isoprostane concentration measured by enzyme immunoassays (ELISA). They demonstrated that within assay variability was small, but there was a considerable within and between day variability for these biomarkers. Their two main conclusions derived from the study are: 1) the cause of relatively low reproducibility of EBC LTB_4 and 8-isoprostane is multi-factorial including both biological and methodological variability; 2) the high level of variability they observed casts doubt on the current EBC methodology used to assess LTB_4 and 8-isoprostane.

The very important contribution of this article to the field is the careful analysis of reproducibility for two biomarkers. It has been shown for some EBC biomarkers that oral contamination during sampling (Gaber et al 2006; Marteus et al 2005), differences in collecting surfaces (Rosias et al 2006), different assay techniques (Huszar et al 2005), and mode of standardization (Kullmann et al 2007) all can contribute to limited reproducibility of results. It is extremely difficult to address all potential methodological biases of ELISA when assessing EBC. Intra-assay variability is relatively easy to assess: one can compare readings of duplicate or triplicate aliquots of the same samples. Determination of inter-assay differences again is best done from the same samples adding aliquots of it to two different plates of the assay. If results of inter-assay reproducibility are good, then different batches of the assay can be used to determine intra-day or intra-week reproducibility. This potential difference needs to be taken into account when using EBC as a source of airway biomarkers (Huszar et al 2005). Determination of intra-week reproducibility then can be performed by 1) keeping the first sample for a week and measuring it together with the second (in this case however the potential effect of storing on mediator concentration needs to be taken into account); or 2) measuring the two samples by different batches of ELISA from the same manufacturer (in this case inter-assay variability complicates interpretation). Although it cannot be established from the study by Borrill and colleagues (2007) what kind of arrangement they used for the determination of intra-day and intra-week variability, their conclusion about the complexity of reproducibility holds very well.

Another issue their study touches upon is the limitations of the usefulness of biomarkers with limited reproducibility. Precise standardizations of traditional lung function tests and exhaled nitric oxide measurements were essential for these variables to provide accurate, well reproducible readings and gain clinical acceptance. EBC biomarkers have to travel through the same path before they can become clinically meaningful tools. The study by Borrill and colleagues (2007) adds important insight into the interpretation of observed differences of EBC LTB_4 and 8-isoprostane and it also emphasizes the need for reproducibility data, not only in healthy subjects, but also in patients. COPD is a disease with fluctuation of airway inflammation and oxidative stress that only partially reflected by clinical symptoms therefore it is reasonable to assume a lower than normal degree of reproducibility of biomarkers representing pathophysiological events of the disease even under stable conditions. Therefore, long-term follow-up studies assessing biomarker reproducibility can help to better understand the dynamics of EBC biomarker profile in this multiplex disease.

I hope you will find the article by Borrill and colleagues (2007) stimulating from both clinical and research point of views.

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Active smoking among asthmatic youth—How concerned we need to be

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There is no doubt that tobacco smoking, whether active or passive, has a harmful effect on health of all individuals. Children are particularly vulnerable to environmental tobacco smoke (ETS), and if exposed have higher incidence of lower respiratory tract illnesses in their early years (Cook and Strachan 1999). Furthermore, it has been suggested that the high levels of ETS exposure during childhood may increase the risk of chronic obstructive respiratory disease in adulthood.

ETS exposure and asthma development

Numerous studies have demonstrated that children of tobacco smoking mothers have higher risk of developing asthma (Martinez et al 1992). Maternal smoking of more than 10 cigarettes a day is associated with higher incidence of asthma, earlier onset of asthma symptoms, and an increased risk of using asthma medication compared with the children of nonsmoking mothers (Weitzman et al 1990). Other data suggest that maternal smoking prenatally and during the child's first year of life is a significant risk factor for the development of wheeze in infancy, but not wheezing starting after the first year of life (Murray et al 2004).

In utero tobacco smoke exposure may be more important than the post-natal exposure. Children born to mothers who have smoked in their pregnancies are more likely to have doctor-diagnosed asthma and current asthma requiring medication use (Gilliland et al 2001). This is an important public health issue, as the US national survey has shown that 16.5% of pregnant women smoke while expecting their babies (Ringel and Evans 2001). However, since the majority of mothers who smoke during pregnancy continue to smoke for the next few years (and children in the first years of life generally spend the majority of their time in the mother's care), it is often difficult to distinguish what effects occur from in utero exposure and what effects are secondary to post-natal ETS exposure. The few studies that managed to carry out analyses which excluded the effect of postnatal ETS exposure showed a significant association between smoking during the pregnancy and recurrent wheezing (Lannero et al 2006).

ETS exposure and asthma severity

Children with established asthma who are exposed to environmental tobacco smoke have more frequent acute exacerbations and poorer lung function (Oldigs et al 1991; Chilmonczyk et al 1993). There appears to be a dose-response relationship, with children both of whose parents smoke suffering more than those where the mother alone smokes, with less respiratory symptoms in those children from families with no ETS exposure (Murray and Morrison 1993).

Active smoking and asthma in adolescence

Adolescence is the period when the majority of smokers start smoking. Active smoking during the childhood and adolescence seriously affects respiratory health by causing decreased lung growth, poorer lung function, increased sputum production, airway obstruction, cough, and shortness of breath (Tyc and Throckmorton-Belzer 2006). A recent study conducted among teenagers has demonstrated that regular smoking in healthy nonallergic adolescents increases the risk of subsequent development of asthma (Gilliland et al 2006). Active tobacco smoking induces lower airway inflammation, and has been associated with diminished response to inhaled and systemic steroids in asthmatic patients.

Active smoking among adolescent asthmatics contributes to the frequency and severity of their asthma symptoms. This was confirmed in a study by Mallol and colleagues (2007) in this issue, which presented the data on smoking habits of asthmatic adolescents in Chile. A further alarming finding of the study was the high prevalence of adolescent female smokers. This appears to mirror the findings from many other countries, in which, even after massive media campaigns,

cigarette smoking remains popular amongst teenagers, and particularly young women.

Why do adolescent asthmatics smoke?

Despite having a chronic respiratory disease, asthmatic adolescents do not restrain themselves from smoking, but have equally high smoking rates as their peers, which raises the question of the possible factors that may predispose them to this form of addictive behavior (Zimlichman et al 2004; Jones et al 2006). Studies have indicated that adolescents who are nonadherent to their asthma treatment are more risk-taking and rebellious, therefore more prone to undertake health-compromising behaviours (Tyc and Throckmorton-Belzer 2006). Factors like exposure to smoking at home and having friends who smoke are likely to trigger smoking behavior in asthmatic adolescents. Children with chronic illness like asthma may also have both disease and treatment-related higher psychosocial distress. School absenteeism and separation from peers due to asthma morbidity may also contribute to smoking behavior by using smoking as a vehicle for reconnecting with their peers. However, these factors can change depending on age, sex, race, and socioeconomic status (Tyc and Throckmorton-Belzer 2006).

How to reduce smoking among adolescents?

This topic has been the subject of a recent review article summarizing the current state of the art (Tonnesen 2002). There has been a huge number of high quality interventional studies conducted among teenagers using different school-based programs targeting smoking behaviour (Thomas and Perera 2006). Although the majority of such trials have shown some benefit on the prevention of active smoking in the short term, there is controversy about longevity of these effects. A study with the longest duration of intervention (lasting 8 years) failed to demonstrate sustained effect of specific intervention (Thomas and Perera 2006).

Recently, governments and public health authorities have been trying to develop new policies which would reduce smoking. One of them has been an increase in cigarette taxes which proved to be effective among women of higher educational level (Ringel and Evans 2001).

Media advertisements have great influence on smoking behavior among young adults. Successful public health campaigns to persuade governments of the need for legislation to end the tobacco advertising campaigns in media resulted in legislations banning all tobacco advertising in the UK (Tobacco Advertising and Promotion Act 2003) and many other developed countries. As a result of an EU Directive, there is a partial ban on tobacco advertising also exists throughout the EU.

However, developing countries largely lack such policies, and as a consequence, tobacco companies continue to market their products. In this era of globalization, the legislations to end the tobacco advertising needs to become global, and having smoke-free schools should be our common goal.

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