Analysis of Exhaled Breath Condensate: a Technique With a Future?

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The greater accessibility of the respiratory system compared with other internal organs means that it should be possible to perform noninvasive assessments of the inflammatory component present in most respiratory diseases. A variety of techniques have been developed that can be used for this purpose, including analysis of bronchoalveolar lavage fluid, exhaled nitric oxide, induced sputum, and exhaled breath condensate. Induced sputum has been used for a number of years in specialized clinics, while analysis of nitric oxide is being consolidated and may enter more widespread use when equipment costs come within reach of more hospitals. Analysis of exhaled breath condensate is another technique, proposed by Russian groups in the early 80s, that has been used extensively in recent years and is well described in the literature.

One of the main theoretical advantages of exhaled breath condensate is the possibility of analyzing a wide range of mediators of inflammation and oxidative stress and even tumor markers—in any individual without a requirement for a high level of patient cooperation. However, reasonable doubts exist regarding the origin of the substances present in the condensate, since although the majority of the sample arises from microdroplets generated at the interface of the lower airway (fluids that cover the airway and alveoli), the possibility of contamination by substances originating in the mouth, oropharynx, or even the upper digestive tract cannot be ruled out. This complicates the interpretation of results, particularly in subjects who are not intubated or tracheotomized.

The technique has been used in children[1] and in mechanically ventilated patients.[2] Differences in the levels of markers of inflammation and oxidative stress have been found in more than a dozen diseases and it has even been suggested that the technique could be sufficiently sensitive to detect differences between smokers and nonsmokers[3,4] or changes in patients who have undergone lobectomy.[5] However, other studies have warned of the marked variability of the results obtained in both healthy subjects and patients with a variety of diseases, with a significant overlap between the populations.[6-8] Significant variability has even been observed in the results obtained in healthy subjects from one day to the next.

Examination of the literature reveals that most of the publications that express the greatest optimism over the use of exhaled breath condensate come from the same research group.[1,4,5] In contrast, Effros et al[7] observed a high degree of variability in the results obtained with this technique and in a recent review drew attention to the problems and artifacts associated with it, including the possibility of oral contamination by ammonia that, according to some authors, interferes with pH measurements[8]; however, other researchers dispute this last point.[9] Effros et al[7] also insisted that it is necessary to assess dilution with a reliable marker that allows comparison of samples, both in multiple samples taken from a single subject on the same or different days and in samples taken from different subjects. In another study, van Hoydonck et al[10] even failed to obtain reliable data on 8-isoprostane and hydrogen peroxide (H_2O_2) in healthy smokers, due to the extent of variability of the results. For all of these reasons, studies are needed that evaluate the use of condensate from the lower airway alone (in intubated or tracheotomized patients) and from patients with digestive diseases (gastroesophageal reflux) to better assess the influence of contamination arising from outside the lower airway.

Efforts to identify a reliable marker of dilution have included the use of ions, urea, and protein concentration, but the results have been variable. Sample conductance may be a useful marker and is currently being assessed. However, initial results indicate a requirement for prior lyophilization of the sample.[8]

Despite the insistence of the group promoting the use of exhaled breath condensate that measurement of solutes is reproducible,[11] other authors report the presence of artifacts and problems associated with the technique, and highlight the need for reliable standardization. This has led the American Thoracic Society and the European Respiratory Society to create a task force to resolve the issue; their report is expected to be released shortly. Furthermore, following an initial period of enthusiasm, many Spanish researchers working in this field have adopted a position of prudence, or even a certain degree of skepticism.

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A group of researchers from the Red Respira respiratory research network (www.redrespira.net) has initiated a series of projects, coordinated by Dr P. Romero Colomer, that are aimed at identifying some useful answers to these problems, adequately standardizing the technique, and allowing it to be applied with sufficient guarantees in future studies.

In this issue of ARCHIVOS DE BRONCONEUMOLOGÍA, de Lema et al[12] report findings that confirm previous data regarding the influence of minute ventilation on the volume of condensate collected; in their study, however, the quality of the sample was not assessed. In a recent report, McCafferty et al[13] demonstrated a direct relationship between minute ventilation and both exhaled water and the volume of condensate collected. In that study, the authors also showed that for the same minute ventilation, the amount of condensate collected increased as tidal volume increased. One of the most interesting points of the study was that the levels of nitrites and proteins were not altered significantly either by changing the minute ventilation or by increasing the tidal volume, a finding that will represent a major step forward towards standardization if other authors succeed in confirming this stability in the measurements. Nevertheless, the data do not agree with the results published slightly earlier by Gessner et al[2] based on a study involving mechanically ventilated patients. Those authors observed an increase in the level of nitrites with increased tidal volume, and reported correlation coefficients of 0.79 for this parameter and 0.6 for the relationship between nitrite level and minute ventilation. Although these findings clearly contradict those reported by McCafferty et al,[13] the latter study was undertaken in healthy subjects and the possibility cannot be ruled out that the volume dependency of these markers only occurs in situations of significant lung damage and not in healthy subjects. Information is also lacking regarding the effect of expiratory flow on the various different condensate markers.

Also in this issue, Bruhn et al[14] report their findings on the measurement of H_2O_2 in exhaled breath condensate. Their study was based on 6 mechanically ventilated patients with adult respiratory distress syndrome, a situation that can be considered optimal for the collection of samples representative of lung damage, and in addition, a lung disease in which the levels of markers of inflammation and oxidative stress are expected to be significantly increased based on previous reports. Although the collection method used (Teflon-coated tube immersed in iced water) is not a standardized system, the amount of liquid obtained in 30 minutes to 1 hour (2-8 mL/h) was consistent with the amounts reported by other authors using commercial equipment (4-12 mL/h). The relationship between the volume collected over time and the minute ventilation coincided with that mentioned above. However, their results on H_2O_2 concentration are extremely worrying. Using a spectrophotometric method that has been widely used by other authors and validating it with a reference sample with or without pretreatment with catalase, the authors found a high degree of variability in the H_2O_2 concentration over time. The most notable finding, however, was that half of the samples displayed inconsistencies between the absorption wavelength and the color of the sample, leading to suspicion of contaminants (microparticles, etc) arising from the bronchial tree itself that could alter the reading. Those findings add another element of doubt regarding the reliability of H_2O_2 measurements used as an indicator of oxidative stress and suggest that significant caution should be taken in interpreting both published results and data arising from future studies, until the various groups mentioned clarify the true situation and the possibilities of this technique.

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