Exhaled Breath Condensates: Analyzing the Expiratory Plume

Richard M. Effros¹, Richard Casaburi¹, Janos Porszasz¹, Edith M. Morales², and Virender Rehan²

¹Department of Medicine and ²Department of Neonatology, LA BioMed/Harbor-UCLA, Torrance, California

The mixture of gases and droplets released during exhalation can be hydrodynamically designated as a “plume.” Traditionally, pulmonologists focused on respiratory gases in these plumes, but recent identification of biomarkers in exhaled breath condensates (EBCs) has initiated vigorous efforts to harness this simple, noninvasive approach for diagnostic purposes (>700 citations in PubMed since 2000).

A typical condenser removes about half of the approximately 45 μ l of liquid water that can theoretically be extracted from each liter of saturated exhaled air. Nearly all of this water evaporates from lung surfaces, but approximately 1/10,000 of the condensate ($\sim$ 4.5 nl) represents droplets released from the airway lining fluid (ALF). The assumption that these droplets are released from airways seems justified because alveolar convection is probably negligible and oral contributions can be minimized. ALF droplets can be generated by detachment from airways or rupture of bubbles from previously occluded airways (1), perhaps associated with audible rales.

Passage of exhaled air through the mouth at a site near the condenser makes it difficult to avoid contamination of EBC with salivary fluid. To ensure that saliva represents less than 10% of ALF volume, less than 10 nl of saliva should be collected in each milliliter of EBC, a daunting requirement. However, this was successfully accomplished in 36 of 40 samples when the mouthpiece was separated from the condenser, as judged by a sufficiently sensitive amylase assay (2).

Nonvolatile biomarkers (e.g., cytokines, ions, and urea) found in EBCs are exclusively derived from ALF droplets (Figure 1) (3–8). Dilution (D) of ALF by water vapor is generally quite variable, but D can be estimated with nonvolatile “dilutional” standard indicators (S), selected to have similar concentrations in ALF and plasma, for example, urea, cations, or conductivity following lyophilization: $[\text{nonvolatile}]_{\text{ALF}} = D \times [\text{nonvolatile}]_{\text{EBC}}$, where $D = [S]_{\text{ALF}}/[S]_{\text{EBC}} = [S]_{\text{plasma}}/[S]_{\text{EBC}}$.

Lyophilization is used to extract water and volatiles (e.g., NH_3 and CO_2) from ice at temperatures below -50°C , and should not be confused with centrifugal evaporation at warmer temperatures, which may denature proteins. The EBC samples can be concentrated by reconstituting the dried samples in smaller volumes of pure water. D must be determined before conclusions

can be reached about concentrations of nonvolatiles in ALF from EBC measurements. For example, increases in EBC nonvolatile concentrations may reflect collection of more ALF droplets rather than increased ALF concentrations, unless it can be shown that EBC dilution remains relatively unchanged. The reliability of specific dilutional indicators can be checked by showing that alternative markers yield similar values of dilution (3) and by documenting that incorporation of dilutional indicators reduces data variability (5–8).

Volatile biomarkers and water directly diffuse as gases into the expiratory airflow from fluid covering *both* *airspaces and airways* (epithelial lining fluid). Volatile solutes with tissue–air partition coefficients above 100 (e.g., acetone) are lost primarily from

Figure 1. Contributions of diffusion and convection to formation of the expiratory plume. Rapid diffusion of water (evaporation) occurs from the fluid lining the surfaces of the airways (e.g., bronchi), the airspaces (e.g., alveoli), and mouth into the expiratory flow. Exhaled water vapor remains in the gas phase until it is cooled and captured as droplets in the condenser. Droplet formation *within the lungs* is largely confined to the airways, where turbulence is encountered. These droplets contain airway lining fluid and are the source of virtually all of the nonvolatile biomarkers, ions, and dilutional indicators that reach the condensate. They are presumably formed by convective detachment from the surface fluid on the airway surfaces or by expulsion of aqueous films spanning airway openings (perhaps causing audible rales). Because the rate of droplet formation is so miniscule compared with the movement of gases from the lungs, transport of nonvolatile constituents is generally much slower than diffusive transport of volatile molecules. Most of the NH_3 and many other gases (e.g., from bacterial metabolism) diffuse into the exhaled stream from the mouth, nose, and upper gastrointestinal tract. High concentrations of these volatile molecules are much more readily maintained at sites (e.g., oral cavity) relatively remote from exchange vessels. Diffusion of respiratory gases occurs primarily between the gas, tissues, and blood in the alveoli, but the site of diffusion of more soluble vapors (such as acetone) is typically greater in the airways.

Supported by the National Institutes of Health (grants HL75405 and HD51857). R.C. occupies the Grancel/Burns Chair in the Rehabilitative Sciences at LA BioMed.

Author Contributions: R.M.E., planning and synthesis of review; R.C., J.P., E.M.M., and V.R., conceptual contributions.

Correspondence and requests for reprints should be addressed to Richard M. Effros, M.D., 1124 West Carson Street, J4, Torrance, CA 90502. E-mail: reffros@labiomed.org

Am J Respir Crit Care Med Vol 185, Iss. 8, pp 803–804, Apr 15, 2012

Copyright © 2012 by the American Thoracic Society

DOI: 10.1164/rccm.201109-1702ED

Internet address: www.atsjournals.org

airways, whereas those with lower coefficients are mostly lost from alveoli (9). Correction for dilution of droplets by water vapor is unnecessary if most of the biomarker is gaseous. However, gas phase measurements may be preferable to EBC studies because the distribution of volatiles between exhaled gas, lung fluid, and condensates can be quite variable. Droplet formation can be minimized by keeping exhaled air warm and measuring partial pressures in the gas phase (e.g., with gas chromatography/mass spectrometry). Volatile biomarkers diffuse more rapidly through lipid membranes separating gas, tissue, and blood compartments of the lungs than nonvolatile indicators and are consequently much more likely to be influenced by plasma concentrations and extrapulmonary inflammation. Because they can also diffuse directly from the oral, nasal, and gastrointestinal tracts into the exhaled breath, increases in EBC concentrations of volatile markers may be unrelated to pulmonary disorders.

Diffusion and convection play additive roles in the transport of partially ionized acids and bases in exhaled air. However, diffusion of uncharged molecules of many volatile acids and bases overwhelms transport of ions, which is limited by the small volume of airway droplets in the EBC. For example, though concentrations of NH_3 are less than 1% those of NH_4^+ in saliva, diffusion of gaseous NH_3 from saliva accounts for most of the NH_4^+ found in the EBC (3, 10), and this transport promotes alkalization of EBC. High salivary concentrations of NH_4^+ and NH_3 are maintained by bacterial degradation of urea in a pool of fluid relatively remote from the circulation. Any NH_3 that is inhaled from the mouth or produced locally in the lungs should rapidly diffuse into the pulmonary and bronchial microvasculature (11).

Early studies suggested that EBC acidification is characteristic of asthma, indicating airway inflammation (12). The fundamental error in most EBC pH studies is the failure to measure the buffering capacities (β) of the ALF and EBC. These are essential for calculating ALF pH. NH_4^+ is overwhelmingly the most abundant cation in EBC, and it is associated with nearly equivalent concentrations of HCO_3^- in samples exposed to room air or 5% CO_2 (3, 10, 13). Addition of weak or strong nonvolatile acids from the ALF generally has comparatively modest effects on EBC pH, which are difficult to detect. EBCs remain relatively alkaline unless acid concentrations in EBCs approach those of NH_4^+ (14), which seldom occurs in oral EBCs. A report that EBC pH is not influenced by NH_4^+ concentrations (15) appears untenable and was not confirmed in subsequent studies (16, 17). Controversy persists regarding the best PCO_2 at which pH should be measured, but conventional flushing with argon does not remove all of the CO_2 , fails to raise pH of EBC to expected levels, and can remove variable amounts of water and other acid-base pairs (10, 16). These problems may explain why EBC acidification was *not* confirmed in a recent large, multicenter study of individuals with asthma (18) and suggest that conventional studies of EBC pH should be abandoned.

In conclusion, EBC studies are best used for investigations of nonvolatile constituents, for example, electrolytes, nongaseous neutral molecules (e.g., sugars and urea), and macromolecules (e.g., cytokines and nucleic acids) in the ALF. In theory, this vast family of potential biomarkers can also be recovered by impaction, bubbling through water, or filtration. The initial enthusiasm regarding EBCs dimmed somewhat when the marked dilution and variability of EBC concentrations were fully appreciated. Although estimates of dilution may reduce variability found in EBC data, progress will require more sensitive and reproducible

assays of all indicators. Nevertheless, it would be difficult to exaggerate the importance of noninvasively measuring airway biomarkers, and investigators should be encouraged to perfect analytical and collection techniques.

Author disclosures are available with the text of this article at www.atsjournals.org.

References

1. Almstrand AC, Bake B, Ljungström E, Larsson P, Bredberg Mirgorodskaya E, Olin ACA. Effect of airway opening on production of exhaled particles. *J Appl Physiol* 2010;108:584–588.
2. Effros RM, Clark KW, Linn WS, Lee EK, Gilliland FD, Rehan VK, Esther CR Jr. Dilution, salivary contamination and reproducibility in exhaled breath condensate studies of bioactive purines. *Am J Respir Crit Care Med* 2011;183:A4468.
3. Effros RM, Biller J, Foss B, Hoagland K, Dunning MB, Castillo D, Bosbous M, Sun F, Shaker R. A simple method for estimating respiratory solute dilution in exhaled breath condensates. *Am J Respir Crit Care Med* 2003;168:1500–1505.
4. Dwyer TM. Sampling airway surface liquid: non-volatiles in the exhaled breath condensate. *Lung* 2004;182:241–250.
5. Baker EH, Clark N, Brennan AL, Fisher DA, Gyl K, Hodson ME, Phillips BJ, Baines DL, Wood DM. Hyperglycemia and cystic fibrosis alter respiratory fluid glucose concentrations estimated by breath condensate analysis. *J Appl Physiol* 2007;102:1969–1975.
6. Esther CR Jr, Boysen G, Olsen BM, Collins LB, Ghio AJ, Swenberg JW, Boucher RC. Mass spectrometric analysis of biomarkers and dilution markers in exhaled breath condensate reveals elevated purines in asthma and cystic fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2009;296:L987–L993.
7. Esther CR Jr, Lazaar AL, Bordonali E, Qaqish B, Boucher RC. Elevated airway purines in COPD. *Chest* 2011;140:954–960.
8. Lazar Z, Cervenak L, Orosz M, Galffy G, Komlosi ZI, Bikov A, Losonczy G, Horvath I. Adenosine triphosphate concentration of exhaled breath condensate in asthma. *Chest* 2010;138:536–542.
9. Anderson JC, Lamm WJE, Hlastala MP. Measuring airway exchange of endogenous acetone using single-exhalation breathing maneuver. *J Appl Physiol* 2006;100:880–889.
10. Effros RM, Casaburi R, Su J, Dunning M, Torday J, Biller J, Shaker R. The effects of volatile salivary acids and bases upon exhaled breath condensate pH. *Am J Respir Crit Care Med* 2006;168:1500–1505.
11. Effros RM, Olson L, Lin W, Audi S, Hogan G, Shaker R, Hoagland K, Foss B. Resistance of the pulmonary epithelium to movement of buffer ions. *Am J Physiol Lung Cell Mol Physiol* 2003;285:L476–L483.
12. Hunt JF, Fang K, Malik R, Snyder A, Malhotra N, Platts-Mills TAE, Gaston B. Endogenous airway acidification: implications for asthma pathophysiology. *Am J Respir Crit Care Med* 2000;161:694–699.
13. Carraro S, Folesani G, Corradi M, Zanconato S, Gaston B, Baraldi E. Acid-base equilibrium in exhaled breath condensate of allergic asthmatic children. *Allergy* 2005;60:476–481.
14. Effros RM, Casaburi R, Porszasz J. Assessment of exhaled breath condensate pH. *Am J Respir Crit Care Med* 2011;183:952.
15. Wells K, Vaughan J, Pajewski TN, Hom S, Ngamtrakulpanit L, Smith A, Nguyen A, Turner R. Exhaled breath condensate pH assays are not influenced by oral ammonia. *Thorax* 2005;60:27–31.
16. Greenwald R, Ferdinands JM, Teague WG. Ionic determinants of exhaled breath condensate pH before and after exercise in adolescent athletes. *Pediatr Pulmonol* 2009;44:768–777.
17. Kullmann T, Barta I, Lázár Z, Szili B, Barát E, Valyon M, Kollai M, Horváth I. Exhaled breath condensate pH standardised for CO_2 partial pressure. *Eur Respir J* 2007;29:496–501.
18. Liu L, Teague WG, Erzurum S, Fitzpatrick A, Mantri S, Dweik RA, Bleecker ER, Meyers D, Busse WW, Calhoun WJ, *et al*. Determinants of exhaled breath condensate pH in a large population with asthma; National Heart, Lung, and Blood Institute Severe Asthma Research Program (SARP). *Chest* 2011;139:328–336.