Exhaled Breath Condensate as a Clinical Diagnostic with a Focus on Acid Reflux Cough

a report by
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Acid reflux cough is a common disease entity encountered not only by sub-specialty respiratory physicians, but increasingly by primary care physicians.[1] It may be relevant in 30–60% of patients with asthma, chronic obstructive pulmonary disease (COPD), and chronic cough, and, although not clear, is probably an important co-morbidity in many other lung diseases.[2] The condition is caused by one or both of the following:

- acid reflux into the esophagus, with stimulation of esophageal vagal nerve branches leading to reflex cough and neurogenic inflammation; and
- higher level, although often very brief episodes, of pharyngeal acid reflux with or without aspiration of acid through the vocal cords.

This latter mechanism contributes to not only cough and neurogenic inflammation, but also direct acid injury to the airway, akin to a burn, or acid-mediated dysfunction of normal cell and protein functions.[3]

Acid reflux cough is not gastroesophageal reflux disease (GERD). GERD results from a pathologic excess of acid reflux into the esophagus, and manifests with esophageal symptoms such as heartburn. In contrast, acid reflux cough can result from infrequent and fleeting episodes of pharyngeal acidity in a process that is fully tolerated by the esophagus, but damaging to the airway.[4] Although acid reflux occurs in both entities, acid reflux cough and GERD can and do occur completely independently of each other.

Diverse respiratory symptoms and signs have been attributed to acid reflux. Cough is the most commonly discussed; however, acid reflux has been implicated in wheezing, choking, pneumonia, laryngitis, globus, airway malacia, sub-glottic stenosis, and multiple other respiratory disorders.[5] There are substantial data supporting its involvement in the major obstructive lung diseases.

Failure of Current Diagnostics

Diagnosis of acid reflux cough is not straightforward. As most patients with acid reflux cough have no history of GERD symptoms, such a history or lack of it provides limited diagnostic assistance.[6] Index of suspicion needs to be high. The reticence of many physicians to prescribe or rely upon esophageal pH probes for this diagnosis is understandable because pH probes:

- measure pH in the wrong organ;
- are uncomfortable;
- are expensive; and
- are usually interpreted based on normal parameters defined for the esophagus, which are irrelevant when dealing with an airway illness.

In contrast to its role in GERD diagnosis, esophageal pH measurement should not be considered a gold standard for acid reflux cough diagnosis. Although pharyngeal pH probe measurements have been used successfully for research,[4] the uncomfortable position of the probes and their tendency to dry out or move off the airway mucosa (causing artifact) make such probes an unattractive option for most clinicians (and even less attractive to most patients).

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2. *Roka R, Rosztoczy A, Izbeki F et al., "Prevalence of respiratory symptoms and diseases associated with gastroesophageal reflux disease",* Digestion *(2005);71(2): pp. 92–96.*
3. *Ricciardolo F L, Gaston B, Hunt J, "Acid stress in the pathology of asthma",* J. Allergy Clin. Immunol. *(2004);113(4): pp. 610–619.*
4. *Eubanks T R, Omelanczuk P, Hillel A et al., "Pharyngeal pH measurements in patients with respiratory symptoms before and during proton pump inhibitor therapy",* Am. J. Surg. *(2001);181(5): pp. 466–470.*
5. *Halstead L A, "Extraesophageal manifestations of GERD: diagnosis and therapy",* Drugs Today *(Barc) (2005);41(suppl. B): pp. 19–26.*
6. *Oelschlager B K, Chang L, Pope C E, 2nd, et al., "Typical GERD symptoms and esophageal pH monitoring are not enough to diagnose pharyngeal reflux",* J. Surg. Res. *(2005);128(1): pp. 55–60.*

Therapeutic trials of proton pump inhibition (PPI) are commonly used to assist in the diagnostic process to help identify acid reflux as a cause of chronic cough. This has entered practice only because of a lack of availability of a more appropriate diagnostic. Therapeutic trials of PPI have the potential to suffer from the following problems of all other such therapeutic challenges:

- lack of specificity and sensitivity;
- long delay before results are available; and
- lack of satisfaction in the diagnostic process and confidence in the result.

Placebo effect and spontaneous resolution of chronic cough symptoms lead to false positive trials, and failure of medication compliance or presence of a concurrent etiology for chronic cough—such as post nasal drip or asthma—lead to false negative trials. When a false positive trial occurs, a patient is left on an expensive medication, potentially indefinitely because, when PPIs are stopped, there can be a substantial and long-lasting rebound gastric acidification that can cause gastrointestinal symptoms to occur, falsely reinforcing an initial incorrect diagnosis.[7]

The concern about concomitant etiologies for chronic cough co-existing with the acid reflux is important because acid reflux is particularly common in asthma and COPD. If cough does not clearly improve with a therapeutic trial of PPIs, it is difficult to tell whether that should be interpreted to rule out acid reflux cough, when it may result from exacerbation of the primary pulmonary disease.

New Diagnostics are Becoming Available

Acid reflux cough is an airway disease and it therefore makes sense that, in most patients, it should be sought by testing airway pH. Over the past five years, gas-standardized measurement of the pH of exhaled breath condensate (EBC) has achieved prominence as the only non-invasive method to assess abnormalities in the acidity of the fluid lining the airways.[8] Any cause of airway acidification can lead to a low EBC pH value,[3] the same way that there are multiple causes for an elevated white blood cell count. An abnormally low EBC pH (normal is above 7.4, based on a normative database of over 400 healthy people)[9] is associated with various airway diseases, including asthma, COPD, and bronchiectasis,[10] as well as with acid reflux.

Airway acidification may be not only an important component of inflammation, but also the initiator of inflammation.[3] Although commonly discussed in the literature as an airway 'inflammometer', EBC pH is best thought of as an airway acidometer, which allows the use of this technology for assessment of various diseases. Using portable EBC collection methods to obtain many samples from a patient over the course of a day, patterns of EBC pH can be used to distinguish the transient, intermittent airway acidification that occurs with acid reflux from the more persistent inflammatory-associated acidification.[11]

As a paradigm shift is needed before it could become generally accepted that asthma and other airway diseases are, at least to some degree, disorders of airway pH homeostasis, EBC pH measurements have been introduced as a clinical diagnostic for an airway acidification process requiring no paradigm shift—acid reflux cough. Another reason why EBC pH assays have been introduced for acid reflux cough is that there are reasonably safe treatment options (PPIs) readily available for acid reflux cough, and these therapies are effective for the condition when targeted correctly. On the other hand, therapies are only now beginning to be designed specifically to target airway acidification occurring as a trigger of or resulting from airway inflammation in asthma. EBC diagnostics will play an important role in the development of the underlying science of airway acidification and in future therapies addressing airway pH homeostasis.

As previously noted, the use of EBC pH makes sense as a tool to evaluate acid reflux cough because it assesses airway pH. More specifically, EBC acidification will occur when the inhaled or exhaled airstream passes an acidic fluid containing volatile water-soluble acids.[12] Thus, the lung, tracheobronchial tree, hypopharynx, and mouth could all be potential contributors to EBC acidification depending on the setting.

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8. *Horvath I, Hunt J, Barnes P J, "Exhaled breath condensate: methodological recommendations and unresolved questions",* Eur. Respir. J. *(2005);26(3): pp. 523–548.*
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11. *Walsh B, "Non-invasive diagnosis of acid reflux cough",* Respiratory Therapy *(2005);1(1): pp. 69–71.*
12. *Hunt J, "Exhaled breath condensate: An evolving tool for noninvasive evaluation of lung disease",* J. Allergy Clin. Immunol. *(2002);110(1): pp. 28–34.*

In acid reflux cough, the author's data and those of others strongly support that pharyngeal acidification is a key component in most patients.[4] The lower esophageal reflux pathway, although present, is an exponentially less effective mechanism of triggering cough than the airway acidification/aspiration mechanism.[13,14] EBC pH measurements can identify the brief transient episodes of acid reflux into the pharynx and lower airway by leveraging this simple fact: acid in the airway triggers cough. Timing EBC collections so that they are performed immediately after a cough (or within 10 minutes) allows for identification of an association of the cough with airway acidification—an association that would likely be missed with random collections of EBC at other times. Rather like a car that rattles, acid reflux cough may not cooperate and be present during a particular visit to the mechanic/physician. A key component of EBC is its ability to be collected at home or at work, multiple times—a valuable feature that allows diagnosis to be made quickly.

Specificity for acid reflux as the cause of EBC acidification found in a patient derives from the multi-sample diagnostic; a profile of cough episodes associated with intermittent low EBC pH values in the setting of a normal baseline pH suggests that airway acidification is arising from acid reflux as opposed to inflammation.

Beyond the Clinic—Current and Future Uses of Exhaled Breath Condensate

Improving Phase III Pharmaceutical Trials Using EBC pH

EBC pH technology may be able to shed light on why studies of PPI therapy for acid reflux cough have met with mixed results. Previous studies have generally been performed in patients with GERD and cough, or with GERD and asthma. In retrospect, this may be found to be an error, because GERD is not the issue. Acid reflux is the issue, and there is an important distinction to be made. Having the correct diagnosis is a prerequisite for a medication to be effective, and it is unrealistic to expect a PPI to treat a cough caused by post-nasal drip or asthma or a hair tickling the tympanic membrane, just because the patient also happens to have unrelated GERD.

Exhaled breath condensate pH is joining exhaled NO as a clinically useful diagnostic, providing objective non-invasive evidence that acid reflux is contributing to a patient's cough.

Systems for Leveraging the Ability of EBC pH to Identify Acid Reflux in Cough

A kit was developed, originally for research purposes, for subjects/patients to collect multiple EBC samples in their homes. The kit consists of eight EBC sample collectors and packaging to mail samples to the laboratory. This system has been tested extensively and is an effective and efficient means of obtaining EBC for subsequent pH assay. It is also convenient for the person providing the samples. Each sample collection takes only five minutes of simple breathing (easier than blowing up a balloon and simpler than playing a flute), and it can all be done at home. This kit has recently been modified and combined with validated gas-standardized EBC pH assay. This assay system is known as Aeriflux (Respiratory Research, Inc., US) and is the only non-invasive acid reflux cough diagnostic.

Non-invasive EBC pH testing applied in carefully designed studies may be used to identify a new study population to enroll in clinical studies of PPI in cough—a population that has airway acidification in association with their cough. Perhaps by enrolling this more appropriate and specific patient population, pharmaceutical companies will be able to obtain regulatory approval for their PPIs for treatment of acid reflux-associated chronic cough, an approval that has been elusive to date, at least in the US. An approved indication will allow pharmaceutical companies to market their PPIs for respiratory

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14. *Lopes F D, Alvarenga G S, Quiles R et al., "Pulmonary responses to tracheal or esophageal acidification in guinea pigs with airway inflammation",* J. Appl. Physiol. *(2002);93(3): pp. 842–847.*

complications of acid reflux, which will be an important step in raising awareness among patients and physicians.

EBC Redox Monitoring

EBC contains much more than acids, and assays for other compounds in EBC may someday provide useful information for the clinical management of individual patients.[8] For example, elevated EBC levels of hydrogen peroxide in patients with respiratory diseases suggest that this oxidant precursor may be contributing to the disease pathology.[15] Levels of 8-isoprostane and ratios of reduced to oxidized glutathione may provide a summation of the oxidative burden and antioxidant availability in the airway and lung.[16] Although they are potentially powerful tools for research into disease mechanisms, the utility of these assays in clinical practice will be somewhat limited until there are effective therapies specifically targeting the redox pathways in the lungs. However, even today, these assays may have important investigative use beyond elucidating pathological mechanisms. For example, there have been several studies of antioxidants for the treatment of airway and lung diseases, and they have had generally disappointing results.

Until now, no methodology to quantify airway oxidant stress has been available, and this lack has prevented the appropriate targeting of antioxidants to study subjects most likely to have positive clinical responses. This failing has likely contributed to the apparent ineffectiveness of the compounds. Just as for PPI therapy in chronic cough, it is necessary to phenotype patients wisely to avoid testing medications on patients in whom a response cannot be expected. EBC redox assays are the tools that seem most useful in this regard.

Augmentation of Exhaled Nitric Oxide Monitoring

Given that many commonly used therapies target inflammation, it does appear to be necessary to have an ability to assess baseline airway inflammation and response to interventions, otherwise physicians are essentially flying blind and guessing. Exhaled nitric oxide (NO) has recently evolved into a clinical tool, serving as a surrogate for airway inflammation. Exhaled NO was extensively reviewed by Philip Silkoff in 2005.[17] Perhaps most useful as a marker of eosinophilic inflammation in asthma, it seems also to have a role in ruling out ciliary dyskinesia. Exhaled NO measurement equipment is available clinically, and data have emerged supporting its potential use in titrating inhaled steroids in asthma.[18] Although these data are somewhat controversial, the intense efforts that have been undertaken by those studying exhaled NO have led to something that helps a patient by allowing physicians to do more than simply guess at optimal steroid dosing.

Supplementation of exhaled gas phase NO testing by measuring aqueous phase nitrogen oxides (NOx) is one potentially emerging EBC parameter.[19] Measuring exhaled NO together with EBC NOx appears to provide a more comprehensive picture of airway NO production, as well as an additional window into oxidant stress in the lung. In this regard, NO is readily oxidized to various higher oxides of nitrogen and if the airway is undergoing oxidant stress, it may be expected that NO will be lower than normal and NOx higher than normal. This is only beginning to be explored, but represents a reasonably likely avenue for success and may allow for improved sensitivity and specificity of exhaled NO inflammometry, and expansion to disease entities such as COPD for which it has not been particularly useful to date.

Conclusion

Exhaled breath condensate pH is joining exhaled NO as a clinically useful diagnostic, providing objective non-invasive evidence that acid reflux is contributing to a patient's cough. Many further potential EBC diagnostics may emerge as translational research is undertaken. The goal of medical research, in the final analysis, is to make patients feel better. The movement of research techniques into the clinical setting is a sometimes difficult but critical component of that process. ■

15. *Loukides S, Horvath I, Wodehouse T et al., "Elevated levels of expired breath hydrogen peroxide in bronchiectasis",* Am. J. Respir. Crit. Care Med. *(1998);158(3): pp. 991–994.*
16. *Montuschi P, Ragazzoni E, Valente S et al., "Validation of 8-isoprostane and prostaglandin E(2) measurements in exhaled breath condensate",* Inflamm. Res. *(2003);52(12): pp. 502–527.*
17. *Silkoff P, "Non-invasive monitoring of airway inflammation with a focus on exhaled nitric oxide",* Business Briefing: US Respiratory Care 2005 *(2005): pp. 77–80.*
18. *Smith A D, Cowan J O, Brassett K P et al., "Use of exhaled nitric oxide measurements to guide treatment in chronic asthma",* N. Engl. J. Med. *(2005);352(21): pp. 2,163–2,173.*
19. *Nguyen T A, Woo-Park J, Hess M et al., "Assaying all of the nitrogen oxides in breath modifies the interpretation of exhaled nitric oxide",* Vascul. Pharmacol. *(2005);43(6): pp. 379–384.*