ATS Workshop Proceedings: Exhaled Nitric Oxide and Nitric Oxide Oxidative Metabolism in Exhaled Breath Condensate

Executive Summary

This official executive summary of an American Thoracic Society Workshop Report was approved by the ATS Board of Directors, September 2005.

BACKGROUND TO WORKSHOP

The field of noninvasive assessment of airway inflammation has developed rapidly since the discoveries in the late 1980s that nitric oxide (NO) is a central biological mediator and, in 1991, that exhaled breath contained NO (1). Accompanying this was the realization that asthma is an inflammatory airway syndrome, best treated by antiinflammatory agents. Subsequently, exhaled NO was found to be high in asthma (2), and to fall after antiinflammatory drugs but not bronchodilators. Today, exhaled NO (F_{ENO}) is progressing from a research tool to clinical application in asthma (3). In the mid-1990s, the field of exhaled breath condensate (EBC) analysis, a biological sample of the airways took off rapidly, opening up another way to sample airway chemistry in a noninvasive manner. Exhaled NO measurement and NO redox assessment in EBC were the focus of an American Thoracic Society (ATS) workshop that was held in Toronto in 2002 and was divided into two sections: (*1*) exhaled and nasal NO and (*2*) lung nitrogen oxide and redox assessment using EBC. The full workshop proceedings can be accessed in the current proceedings of the ATS.

EXHALED NO

The workshop commenced with updates on the measurement of F_{ENO} since the previous 1998 ATS workshop, which resulted in the 1999 ATS statement on exhaled and nasal NO measurement (4). Several new sensor technologies to detect NO were presented, and experience with the 1999 online and offline exhaled NO measurement recommendations was reviewed. In general, the methods recommended in 1999 had met with success and acceptance, although several minor modifications were adopted.

For online F_{ENO}, the need to inspire to total lung capacity (TLC) was relaxed to a near-TLC inspiration for patients who could not inspire fully. Also, as F_{ENO} is so reproducible, it was believed that two reproducible measures were adequate instead of the previous requirement for three reproducible measures. The online flow rate of 50 ml/s was believed to be acceptable and reproducible for both adults and children. The section on F_{ENO} plateau definition was revised to make defining this plateau clearer.

For offline exhaled NO methods, there was discussion around the 1999 flow rate of 350 ml/s, which is much higher than the online method (50 ml/s) (4). It was agreed, however, that investigators could use other flow rates for offline measurement as long as this is reported in manuscripts. Pediatric exhaled NO measurement was reviewed only briefly as there is a recent ATS/European Respiratory Society (ERS) task force that published recommendations in 2002 (5).

Clinical application of exhaled NO was thought to be limited to asthma at this stage, and the evidence for the use of this marker in asthma was reviewed.

Techniques of nasal NO measurement were discussed, with a general consensus that the 1999 nasal NO recommendations were adequate for most research purposes (4). Regarding the clinical application of nasal NO, it was decided that the only promising application was in screening for primary ciliary dyskinesia syndromes (6), and that a simple widely applicable method for this application should be developed.

The development of NO excretion models for the lung with a description of flow-independent NO exchange parameters was reviewed (7). Finally, approaches to NO measurement in the ventilated patient, such as the online single-breath method (8), were presented and discussed by a separate breakout group.

One purpose of the workshop was to revise and update the 1999 ATS exhaled and nasal NO recommendations. This was achieved at a final session and the 2005 ATS/ERS statement on exhaled and nasal NO measurement has now appeared in the *AJRCCM* (9), and can be accessed from the ERS website and was accompanied by an editorial (10).

LUNG NITROGEN OXIDE AND REDOX ASSESSMENT USING EBC

Background

This workshop focused on the potential of EBC to provide information regarding lung nitrogen and oxygen redox balance. Methods by which measurements of higher oxides of nitrogen (e.g., nitrite [NO_2^- and NO_3^-]) in EBC, as well as oxidants such as hydrogen peroxide (H_2O_2), and acidity that could complement the more established exhaled NO test were presented.

Anatomic Source of EBC Components

Oral collections of EBC are the most commonly used, although nasal and endotracheal collections are increasingly reported. All nonvolatile compounds found in EBC come from either the droplets/particles evolved from the airway lining fluid (ALF; oropharynx, trachea, bronchi, alveolae) or are reaction products of volatiles that enter EBC from gas phase. Although endotracheal collections of EBC exclude oropharyngeal contribution, most studies of EBC involve oral collections. Even a small contribution of saliva to an EBC sample may be relevant, as the concentrations in EBC of nonvolatile substances are generally very low (micromolar range or below). There are as yet no data regarding the extent to which aerosolization of alveolar fluid

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contributes to EBC. Because alveolar fluid moves toward the proximal airways, there is likely to be some alveolar representation in EBC. Indeed, the first studies of EBC involved surfactant (11).

Nitrogen Oxides

Deviations of EBC nitrogen oxide (NOx) concentrations, or ratios among them, may reflect the oxidative and NOx conditions of the airway, including not just formation of NO, but all the various inorganic, eukaryotic, and prokaryotic reactions that occur around NO (12). These assays may then reflect NO synthase (NOS) activity, airway inflammation, innate immune responses, bacterial burdens, airway pH, and nitrergic neurotransmission. Importantly, assays for the higher oxides of nitrogen complement knowledge gained from exhaled NO assays. For example, although the cystic fibrosis lung has prominent inflammation, exhaled NO levels are generally low. However EBC NOx are found at high concentrations (13), perhaps suggesting that the NO, although indeed being produced in larger amounts, is being oxidized. Another appeal of EBC NOx is less intuitive, but intriguing, as data support that EBC NO_2^- levels provide knowledge regarding lung overexpansion in intubated subjects (14).

EBC pH Assays

Only recently has progress been made in understanding the role of airway pH in respiratory illness. One theoretic underpinning for studying EBC pH is that volatile airway acids could be trapped in EBC, and that they would be exhaled to a greater extent from an acidic source fluid. EBC pH has been found to be low in many respiratory diseases, and increased attention has been given to airway acid–base balance. EBC pH assays have undergone more extensive validation experiments than many other assays in EBC. Although with oral collections EBC will always in part represent (or be contaminated by) the oropharynx, a relevant contribution of orally derived ammonia to the EBC pH has not been identified in specific experiments. Gas standardization or deaeration of EBC prior to measurement of pH provides different information than measurement of pH immediately after collection. Both methodologies may have advantages. Gastroesophageal reflux of acidic fluid with or without microaspiration into the trachea is one potential mechanism of EBC acidification that has been considered, and is under active investigation in several laboratories (15). Alternate explanations for EBC acidification include lower airway acid production by numerous described pathways. In all likelihood, any process that acidifies any level of the airway could lead to EBC acidification if anions present in the fluid become protonated and volatilized.

Interactions of pH and NOx

NO produced in the lower airway may be exhaled but could also undergo chemical change, including oxidation to NOx. As noted, these nonvolatile NOx can be exhaled and serve as a marker of NOS activity that complements and expands on the information provided by exhaled NO. Experimental acidification of the ALF is known to lead to rapid and substantial increases in exhaled NO (16), likely through protonation and breakdown of NO_2^-. However, an association between EBC pH and exhaled NO is not generally identified in clinical studies (13). Acid and NOx together increase the likelihood of formation of S-nitrosothiols as well as nitrated proteins, which affect diverse aspects of lung physiology and immunology.

Hydrogen Peroxide

EBC has provided the strongest evidence to date of oxidative disturbance in lung diseases. With more supportive validation studies than most other EBC markers, H_2O_2 has received extensive attention (17). The flow dependence of EBC H_2O_2 suggests that it arrives in EBC partially in the gas phase, and may arise from the airway wall, similar to exhaled NO (18). The reactivity of H_2O_2 makes it necessary to handle specimens expeditiously.

Evidence of Oxidation by Measurement of Larger Molecules in EBC

Larger molecules exist in EBC (e.g, lipids). Evidence of lipid oxidation can be derived from the presence of such substances as malonaldehyde and 8-isoprostane, which have been found to be particularly informative in diverse respiratory and nonrespiratory disease states. Glutathione, both in reduced and oxidized form, has been identified in EBC and the levels or ratios of these compounds are particularly interesting in assessing the overall redox balance of the lung more comprehensively (19).

Still clouded by uncertainties regarding anatomic sources and complex chemistries, EBC nonetheless allows us to gain cautious insights into ALF constituents in disease states and in health. Lung redox information has become obtainable through this technology, through assays of pH, nitrogen oxides, hydrogen peroxide, glutathione, and markers of the presence of disturbances in these compounds, such as nitrotyrosine, isoprostane, and aldehydes. EBC has potential to complement F_{ENO} to more fully elucidate the nitrogen oxide chemistry of the airways and lung. As a marker of disease activity, EBC assays may monitor acid stress, oxidative stress, and inflammation, separate but often-related entities to which many of our therapies are directed.

THIS OFFICIAL EXECUTIVE SUMMARY OF AN ATS WORKSHOP PROCEEDINGS WAS DEVELOPED BY AN AD HOC SUBCOMMITTEE OF THE ASSEMBLY ON ALLERGY, IMMUNOLOGY, AND INFLAMMATION.

Writing Committee
PHILIP E. SILKOFF, M.D.
JOHN F. HUNT, M.D.

Conflict of Interest Statement: P.E.S. is currently a full-time employee of AstraZeneca but this bears no relationship or conflict of interest to this document; previously, he was a paid consultant for Ionics-Sievers Instruments and Aerocrine AB, manufacturers of exhaled NO meters; he receives royalties from Ionics-Sievers Instruments and Aperon Biosystems for licensed patents. J.H. is a cofounder and substantial shareholder of Respiratory Research, Inc., which designs and manufactures exhaled breath condensate collection equipment and has licensed exhaled breath condensate pH and other assays from the University of Virginia; he is an inventor of exhaled breath condensate nitrogen oxide and pH assays.

Workshop Participants
PHILIP E. SILKOFF, M.D., United States (*Chair*)
SERPIL C. ERZURUM, M.D., United States (*Chair*)
JOHN F. HUNT, M.D., United States (*Chair*)
KJELL H. ALVING, Ph.D., Sweden
EUGENIO BARALDI, M.D., Italy
JOSE M. CHATKIN, M.D., Brazil
MASSIMO CORRADI, M.D., Italy
AARON DEYKIN, M.D., United States
RAED A. DWEIK, M.D., United States
RICHARD EFFROS, M.D., United States
W. MICHAEL FOSTER, PH.D., United States
BEN GASTON, M.D., United States
STEVEN C. GEORGE, M.D. ,PH.D., United States
CHRISTIAN GESSNER, M.D., Germany
GIANFRANCO GIUBILEO, M.D., Italy
MICHAEL GOLDMAN, M.D., United States
CARLOS GUTIERREZ, M.D., Canada
MARIEANN HOGMAN, Ph.D., Sweden
JENS HOHLFELD, M.D., Germany
OLAF HOLZ, Germany
ILDIKO HORVATH, M.D., Hungary
DANIEL W. LASKOWSKI, R.P.F.T., United States
JON O. LUNDBERG, PH.D., Sweden
NANDOR MARCZIN, M.D., PH.D., United Kingdom
KAREN MCRAE, M.D., Canada

Sanjay Mehta, M.D., Canada
Anna-Carin Olin, M.D., Sweden
Solbert Permutt, M.D., United States
Anthony Redington, M.D., United Kingdom
Terrence Risby, Ph.D., United States
Richard A. Robbins, M.D., United States
Jigme Sethi, M.D., United States
Robert S. Tepper, M.D., Ph.D., United States
Eddie Weitzberg, M.D., Sweden
Hubert Wirtz, M.D., Germany

Company Representatives

Aerocrine AB, Sweden: Mats Carlson; Aerocrine, Inc., United States: Trevor Bourke, Ian McLeod; Eco Physics, United States: Tom McKarns; Eco Medics, Switzerland: Dirk Wendt; EKIPS Technologies, United States: Patrick McCann; FILT Lungen- und Thoraxdiagnostik, Germany: Gunther Becher; Ionics Instruments, United States: Ric Hutte; Jaeger, Germany: Werner R. Steinhaeusser, Gunther Becher; Methapharm, Canada: Karen Micula

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