Exhaled Breath Analysis and Sleep

Giovanna E. Carpagnano, M.D.

Institute of Respiratory Disease, Department of Medical and Occupational Sciences, University of Foggia, Italy

It is currently estimated that the economic burden for obstructive sleep apnea syndrome (OSAS) cases not coming to medical attention is steadily increasing, thus making OSAS a major public health concern. For its increasing incidence among the common population, the interest of researchers and clinicians has been recently directed to the study of pathological mechanisms underlying sleep disorders. Current opinion is that airway inflammation and oxidative stress play a crucial role in the pathophysiology of OSAS. Recently there has been increasing interest in the investigation of lungs by non-invasive means measuring the exhaled breath volatile mediators, such as nitric oxide (NO), carbon monoxide (CO), ethane and pentane and finally the non-volatile substances in the liquid phase of exhalate, termed breath condensate. The non-invasiveness of these techniques for the study of airways

affected by different respiratory disorders and among those, the OSAS, makes these ideally suited for the evaluation and serial monitoring of patients. Notwithstanding the increasing number of scientific contributions on the use of the exhaled markers in sleep disorders, at the moment, their use is not completely suitable for clinical application. An important contribution to the increase of our knowledge on exhaled markers and for their possible concrete application in clinical practice may come from future studies using proteomics, genomics and metabolomics. In this review, we focus on exhaled breath analysis giving an update on its general aspects, its application in OSAS, and finally its actual clinical applicability and areas for future direction.

Citation: Carpagnano GE. Exhaled breath analysis and sleep. *J Clin Sleep Med* 2011;7(5):Supplement S34-S37.

Obstructive sleep apnea syndrome (OSAS) occurs in 9% of men and 4% of women in the middle-aged American population, and it is characterized by recurrent episodes of upper airway collapse that lead to significant hypoxemia and disturbed sleep architecture.¹ Current evidence indicates that airway inflammation and oxidative stress are both important in the pathophysiology of OSAS. Notwithstanding the key role these events play in obstructive sleep apnea, their monitoring is not included in the current management of this disease.

The direct sampling of airways is today done using quite invasive techniques, such as bronchoscopy with bronchial lavage and biopsy. Recently there has been increasing interest in the non-invasive methods that allow the study of airways such as the exhaled breath volatile mediators and the exhaled breath condensate. The non-invasiveness of these techniques makes these ideally suitable for the evaluation and serial monitoring of obstructive sleep apnea patients. The aim of this review is to outline current knowledge on the exhaled breath analysis highlighting its potential clinical applications in sleep apnea.

Exhaled Breath Volatile Mediators

Exhaled nitric oxide (eNO)

The first exhaled inflammatory marker used in the study of airways of OSAS patients was exhaled NO, a reactive molecule produced by nitric oxide synthase (NOS) enzymes in a variety of cells, such as macrophages and epithelial cells, under the stimulus of cytokines. This molecule is released in the circulation where it acts both as vasodilator and as chemotactic agent for neutrophils, and in airways as exhaled gas.

A task force of the European Respiratory Society (ERS) published European recommendations for exhaled NO measurement in 1997.² Later the American Thoracic Society (ATS) published a statement in 1999, which was updated in 2005.³

Various studies have investigated eNO in OSAS. The first one was performed by Olopade et al. who found an increase of nasal, but not oral, eNO, after sleep in patients suffering from moderate-severe OSAS.⁴ These results contrasted with those of Agustí et al., who did not report any eNO differences in OSAS patients with respect to the controls, although the authors of this study did not analyze eNO before or after sleep.⁵ In both studies the role of obesity was not clearly investigated. Considering that obesity is one of the principal risk factors for OSAS and that it can also have a role in the development of inflammation, it is worth noting that recently eNO has been evaluated even in obese non-OSAS subjects and in non-obese OSAS subjects.

In children, high levels of eNO were found in simple snoring and OSAS compared to the control group. In this case, thin and obese patients without any sleep disorder had the same eNO level.⁶ On the contrary, in adult patients, it was demonstrated that eNO was not always increased in patients with OSAS, although even in obese subjects without sleep disorders a positive correlation was observed between eNO and body mass index (BMI) as well as with apnea hypopnea index (AHI).^{7,8} This finding suggests that obesity could increase not only systemic inflammation but also pulmonary inflammation. Although the relation between obesity and systemic inflammation is well known, the link between obesity and pulmonary inflammation still remains unclear. Certainly adipose tissue is an important source of cytokines, collectively called adipokines, which are implicated in systemic

inflammation, and probably these adipokines could play a fundamental role in increasing pulmonary inflammation too.

While many studies have demonstrated that the plasma level of NO increases after CPAP, few data are available about eNO. One study investigated eNO after CPAP therapy and showed that there was an increase of alveolar eNO,⁹ but this was evaluated only after 2 nights of therapy and thus there are no data relating to the long-term effect of CPAP. On the contrary, a recent large-scale study showed that eNO was higher in OSAS patients with respect to the control normal weight group and that after 1 month of CPAP no change was obtained.¹⁰ The contrasting results among these studies maybe due to different sample methods.

As regards to the use of multiple flow-rate measurements of NO fractionating from alveoli and bronchi, Foresi et al.¹¹ demonstrated that there are some differences between bronchial eNO and alveolar eNO. In fact, in this study, while bronchial eNO was the same in OSAS patients and in control group, alveolar eNO was lower in OSAS.¹¹

In conclusion, eNO has been found to be increased in OSAS as in other pulmonary inflammatory diseases, even though the mechanisms involved in eNO production in OSAS remain complex.

Exhaled pentane

Another exhaled breath volatile mediator studied in OSAS is pentane. Pentane is a volatile hydrocarbon, product of lipid hydroperoxide decomposition that can be measured in orally and nasally exhaled air.¹² Exhaled pentane has been suggested as a simple, objective, non-invasive marker of inflammation.

Exhaled nasal and oral pentane levels were measured before and after sleep in patients with moderate-severe OSAS and were found to be significantly higher after sleep in OSAS, although there were no significant differences in exhaled nasal and oral pentane levels before and after sleep in the control subjects.⁴ No other studies, except the one by Olopade,⁴ are available to date on the use of exhaled pentane in OSAS.

Exhaled carbon monoxide

Exhaled carbon monoxide is mainly produced by enzyme heme oxygenase (HO) and has important roles in many physiological and pathological conditions relating to events determining vasomotion, cell growth, apoptosis and inflammation. Monitoring exhaled CO concentrations could represent a modern, simple, non-invasive, and well reproducible method for the diagnosis and observation of both the progression and severity and the response to therapy of many respiratory diseases.¹³ Only one study exists on the measurement of exhaled CO, as an inflammatory surrogate marker, in sleep apnea patients. The authors of this study showed that there are higher concentrations of exhaled CO in patients with OSAS in comparison with non-apneic (obese and non-obese) subjects. Furthermore, they found that exhaled CO is positively correlated with the severity of OSAS and that a one-month administration of CPAP reduces its concentration, demonstrating an improvement of airway inflammation and oxidative stress.¹¹

Clinical applicability and areas for future direction for exhaled breath volatile mediators

At the moment, the use of exhaled breath volatile mediators is not suitable for clinical application. Future studies are

needed to better explore these markers of inflammation in sleep apnea. Areas for future direction might include the definition of the exact significance of the exhaled gases in the sleep apnea syndrome and in obesity, their influence mediated by circadian rhythm and their study before and after CPAP, or other mechanical and surgical therapies.

Exhaled Breath Condensate

With more than 600 publications available on the use of EBC in pneumology, it is important to underline that the number of publications is increasing exponentially year by year. Considerable interest has recently been devoted to the measurement of non-volatile inflammatory markers in the exhaled breath condensate (EBC) of subjects affected by obstructive sleep apnea syndrome as demonstrated by more than 20 scientific contributions available on PubMed.

A set of general methodological recommendations for oral EBC collection, as well as the limits of this collection method have been published by an expert panel. The panel also recommends areas of further research for this sampling method.¹⁴

EBC presents several advantages that justify its recent diffusion in research and clinical setting: above all there are features such as its non-invasiveness, safety, simplicity, inexpensiveness of sample collection, easy acceptability by subjects and by ethical committees and last its repeatability. The interest in EBC for the study of mechanisms driving sleep apnea has been directed to the study of several inflammatory, oxidative stress and vascular markers.

Inflammatory markers measured in EBC

Among inflammatory markers measured in EBC of OSAS patients, pH is certainly the most studied.^{11,15,16} In all studies on pH, this marker was lower in EBC of OSAS patients compared to controls, correlated with AHI, and increased after one month of daily use of CPAP. Another largely studied inflammatory marker in OSAS is leukotriene (LT)-B₄. LTB₄ is present in high concentrations in EBC of OSAS patients and is significantly reduced after three months of CPAP.¹⁷ Higher exhaled LTB₄ levels were also described in children with an AHI > 5 and in simple snoring.^{18,19}

Among the non soluble inflammatory markers measurable in EBC, nitrite (NO₂⁻), nitrate (NO₃⁻) and 3-nitrotyrosine have been studied in OSAS.²⁰⁻²² To our knowledge, only one article has investigated levels of nitrite/nitrate in EBC of OSAS patients, reporting high levels of these markers¹¹ that are reduced after CPAP treatment. The author also reported a positive correlation between NOx levels and disease severity but not with obesity, which seems not to have a role in airway production of nitrite, nitrate (NO₃⁻) and 3-nitrotyrosine.

Tumor necrosis factor (TNF) α ; is a cytokine produced by different cells which plays a fundamental role in the development of inflammatory status. Two recent articles, both by Li and co-workers,^{23,24} investigated TNF- α ; in the EBC of OSAS patients; in both cases not only was TNF- α ; values higher than in the control group, but it was also correlated with the severity of the disease. The treatment of sleep apnea with CPAP, surgery or oral appliances always found a significant decrease of different cytokines in EBC, including TNF- α ;. Also IL-6, a recognized marker of neutrophilic inflammation, has been

studied in the EBC of OSAS patients and has been reported to be elevated.^{11,16,23,25}

Oxidative stress markers measured in EBC

Among markers of oxidative stress measured in EBC of OSAS patients, the most important is certainly the 8-Isoprostane (8-iso), a prostaglandin (PG)-F₂-like compound belonging to the F₂ isoprostane class that is produced in vivo by the free radical-catalyzed peroxidation of arachidonic acid 26. Several studies reported higher levels of 8-Iso in EBC of OSAS patients than in those who were obese without OSAS.^{16,27} All studies showed a positive correlation between the severity of disease, expressed as the AHI, and the level of exhaled 8-Iso. Two different studies demonstrated that treatment with CPAP (2 d and 2 mo of treatment) significantly decreases the exhaled 8-Iso level.^{11,23} As in the case of adults, the level of EBC 8-Iso seems to be high in children with OSAS. Another oxidative stress marker analyzed in EBC of OSA patients and found to be elevated is hydrogen peroxide (H₂O₂).¹¹

Other markers measured in EBC

Recent data²⁸ have shown an increase of the intercellular adhesion molecule (ICAM-1) in the EBC of OSAS patients, demonstrating that it both promotes leukocyte migration and sustains local inflammation in airways. There is a wide-scale need for more studies to better clarify the link between exhaled ICAM and OSAS. Also, erythropoietin has been investigated in EBC of OSAS patients although Schumann and co-workers did not report differences among erythropoietin EBC levels in OSAS, COPD and healthy subjects.²⁹

Clinical applicability and areas for future direction for exhaled breath condensate

Notwithstanding the increasing number of scientific contributions on the use of the EBC at this moment in time, this method still remains confined to the field of research because none of the biomarkers in EBC have been validated sufficiently for clinical use. However, although the use of the EBC has not reached clinical applicability, it is important to underline that studies on this field have been important as they have improved understanding of the physiopathology of several respiratory disorders and their systemic consequences. Unfortunately, unlike exhaled NO standardization, which required a relatively short time to achieve owing to the fact that hundreds of investigators focused on one molecule, for EBC the period of work is longer because standardization might be directed to each and any individual biomarker. It is the very diversity of EBC itself that has prevented it from achieving clinical applicability so far. An important contribution to the increase of our knowledge in this area and its possible direct application to clinical practice may come from future studies using this method in conjunction with proteomics, genomics and metabolomics. There is only one contribution using proteomics that exists in which the subjects were smokers who were at risk for developing COPD. More studies exist using genomics beginning in 2005 when human DNA was extracted from EBC, which as a result, has become a useful sample for biomolecular analysis. Subsequently, the analysis of polymorphisms and microsatellite alterations has been done in the EBC of patients affected by lung cancer

and asthma. Recently our group was involved in the study of microsatellite alterations in genes that regulate the TNF- α , resistin and uncoupling protein expression in obese subjects and in those affected by sleep apnea (unpublished data).

Great expectations are awaited from the metabolomic applications connected to EBC. In this regard, two studies are available on the EBC of asthmatic and COPD subjects. However, considering the potentiality of this application, a study on sleep apnea is likely forthcoming in the near future.

As regards the possibility to study gene expression, at the moment it is not feasible. No research group has been able to extract RNA from EBC. That said, discoveries are quick to arise in this field and so we are likely to be pleasantly surprised where this method could lead to, by means of expanding the portfolio of non-invasive assays for the multiple coexisting pathological mechanisms underlying respiratory disorders, such as obstructive sleep apnea syndrome.

We think that our knowledge concerning airway inflammation in OSAS is still relatively scanty. This is particularly true with respect to physiopathologic mechanisms, as well as with the links to systemic inflammation, and regarding the latter's role in the development of the comorbidity associated with OSAS, especially cardiovascular diseases. Non-invasive methods such as EBC will allow us to better understand these phenomena, and so it is necessary to encourage research in these directions.

REFERENCES

1. Yaggi HK, Strohl KP. Adult obstructive sleep apnea/hypoapnea syndrome: definitions, risk factors and pathogenesis. *Chest in Chest Medicine* 2010;31:179–86.
2. Kharitonov S, Alving K, Barnes PJ. Exhaled and nasal nitric oxide measurements: recommendations. The European Respiratory Society Task Force. *Eur Respir J* 1997;10:1683–93.
3. American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric Oxide, 2005. *Am J Respir Crit Care Med* 2005;171:912–30.
4. Olopade CO, Christon JA, Zakkar M, et al. Exhaled pentane and nitric oxide levels in patients with obstructive sleep apnea. *Chest* 1997;111:1500–4.
5. Agustí AG, Barbe F, Togores B. Exhaled nitric oxide in patients with sleep apnea. *Sleep* 1999;22:231–5.
6. Verhulst SL, Aerts L, Jacobs S, et al. Sleep-Disordered Breathing, Obesity, and Airway Inflammation in Children and Adolescents. *Chest* 2008;134:1169–75.
7. Depalo A, Carpagnano GE, Spanevello A, et al. Exhaled NO and iNOS expression in sputum cells of healthy, obese and OSA subjects. *J Intern Med* 2008;263:70–8.
8. Carpagnano GE, Spanevello A, Sabato R, et al. Exhaled pH, exhaled nitric oxide, and induced sputum cellularity in obese patients with obstructive sleep apnea syndrome. *Transl Res* 2008;151:45–50.
9. Petrosyan M, Perraki E, Simoes D, et al. Exhaled breath markers in patients with obstructive sleep apnea. *Sleep Breath* 2008;12:207–15.
10. Lim LH, Wagner EM. Airway Distension Promotes Leukocyte Recruitment in Rat Tracheal Circulation. *Am J Respir Crit Care Med* 2003;168:1068–74.
11. Foresi A, Leone C, Olivieri D, Cremona G. Alveolar-Derived Exhaled Nitric Oxide Is Reduced in Obstructive Sleep Apnea Syndrome. *Chest* 2007;132:860–7.
12. Paredi P, Kharitonov SA, Leak D, Ward S, Cramer D, Barnes PJ. Exhaled ethane, a marker of lipid peroxidation, is elevated in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2000;162(2 Pt 1):369–73.
13. Babusikova E, Jesenak M, Durdik P, Dobrota D, Banovcin P. Exhaled carbon monoxide as a new marker of respiratory diseases in children. *J Physiol Pharmacol* 2008;59(Suppl 6):9–17.
14. Horváth I, Hunt J, Barnes PJ, Alving K, Antczak A, et al. ATS/ERS Task Force on Exhaled Breath Condensate. Exhaled breath condensate: methodological recommendations and unresolved questions. *Eur Respir J* 2005;26:523–548.

15. Antonopoulou S, Loukides S, Papatheodorou G, Roussos C, Alchanatis M. Airway inflammation in obstructive sleep apnea: Is leptin the missing link? *Respir Med* 2008;102:1399–405.
16. Carpagnano GE, Spanevello A, Sabato R, Depalo A, Turchiarelli V, Foschino Barbaro MP. Exhaled pH, exhaled nitric oxide, and induced sputum cellularity in obese patients with obstructive sleep apnea syndrome. *Transl Res* 2008;151:45–50.
17. Lefebvre B, Pepin JL, Baguet JP, et al. Leukotriene B4: early mediator of atherosclerosis in obstructive sleep apnoea? *Eur Respir J* 2008;32:113–20.
18. Goldbart AD, Krishna J, Li RC, Serpero LD, Gozal D. Inflammatory mediators in exhaled breath condensate of children with obstructive sleep apnea syndrome. *Chest* 2006;130:143–8.
19. Goldbart AD, Goldman JL, Veling MC, Gozal D. Leukotriene modifier therapy for mild sleep-disordered breathing in children. *Am J Respir Crit Care Med* 2005;172:364–70.
20. Schulz R, Schmidt D, Blum A, et al. Decreased plasma levels of nitric oxide derivatives in obstructive sleep apnoea: response to CPAP therapy. *Thorax* 2000;55:1046–51.
21. Ip MS, Lam B, Chan LY, et al. Circulating nitric oxide is suppressed in obstructive sleep apnea and is reversed by nasal continuous positive airway pressure. *Am J Respir Crit Care Med* 2000;162:2166–71.
22. Alonso-Fernández A, García-Río F, Arias MA, et al. Effects of CPAP on oxidative stress and nitrate efficiency in sleep apnoea: a randomised trial. *Thorax* 2009;64:581–6.
23. Li Y, Chongsuvivatwong V, Geater A, Liu A. Are Biomarker Levels a Good Follow-Up Tool for Evaluating Obstructive Sleep Apnea Syndrome Treatments? *Respiration* 2008;76:317–23.
24. Li Y, Chongsuvivatwong V, Geater A, Liu A. Exhaled breath condensate cytokine level as a diagnostic tool for obstructive sleep apnea syndrome. *Sleep Med* 2009;10:95–103.
25. Minoguchi K, Yokoe T, Tanaka A, et al. Association between lipid peroxidation and inflammation in obstructive sleep apnoea. *Eur Respir J* 2006;28:378–85.
26. Morrow JD, Awad JA, Boss HJ, Blair IA, Roberts LJ. Non-cyclooxygenase derived prostanoids (F2-isoprostanes) are formed in situ on phospholipids. *Proc Nat Acad Sci USA* 1992;89:721–5.
27. Carpagnano GE, Kharitonov SA, Resta O, et al. Increased 8-isoprostane and interleukin-6 in breath condensate of obstructive sleep apnea patients. *Chest* 2002;122:1162–1167.
28. Carpagnano GE, Spanevello A, Sabato R, et al. Systemic and airway inflammation in sleep apnea and obesity: the role of ICAM-1 and IL-8. *Transl Res* 2010;155:35–43.
29. Schumann C, Triantafyllou K, Krueger S, et al. Detection of erythropoietin in exhaled breath condensate of nonhypoxic subjects using a multiplex bead array. *Mediators Inflamm* 2006; 2006;18,061.

ACKNOWLEDGMENTS

Editing of the conference proceedings supported by HL104874.

CORRESPONDENCE

Address correspondence to: Dr. Giovanna Elisiana Carpagnano, Institute of Respiratory Disease, University of Foggia, Ospedale D'Avanzo, Via degli Aviatori 1, 71100 Foggia, Italy; Tel: +39 0881 733143; Fax: +390881733158; E-mail: ge.carpagnano@unifg.it

DISCLOSURE STATEMENT

Dr. Carpagnano has indicated no financial conflicts of interest.