**Institution:** Imperial College London

**Unit of Assessment:** 01 Clinical Medicine

**Title of case study:** Exhaled Nitric Oxide as a Non-Invasive Biomarker of Lung Inflammation

**1. Summary of the impact** (indicative maximum 100 words)

In 1994, Professor Barnes and colleagues at Imperial College showed that nitric oxide (NO) concentrations were increased in the breath of asthmatic patients compared to non-asthmatic controls and were reduced after treatment with inhaled steroids. They subsequently demonstrated that exhaled NO ($FE_{NO}$) could be reliably measured in the clinic, was correlated with eosinophilic airway inflammation in asthma, was increased with airway inflammation and decreased when asthma was controlled. Exhaled NO has subsequently been shown by many investigators to be a useful non-invasive biomarker of airway inflammation in asthma and to improve clinical management in selected patients. They demonstrated that nasal NO is very low in patients with primary ciliary dyskinesia and is now recommended worldwide as a diagnostic test for this disease as it is a much easier method than previously available tests.

**2. Underpinning research** (indicative maximum 500 words)

Key Imperial College London researchers:
Professor Peter Barnes, Professor of Thoracic Medicine (1985-present)
Dr Sergei Kharitonov, Clinical Lecturer (1993-2011)
Dr Paolo Paredi, Clinical Research Fellow (2000-present)
Dr Ron Logan-Sinclair, Medical Engineer (1990-2008)

In 1994, Professor Barnes and colleagues at Imperial demonstrated that there was an increase in NO in exhaled breath of asthmatic patients compared to normal subjects and that the levels of exhaled NO were reduced in patients treated with inhaled corticosteroids (1). This research was based on the finding that inducible NO synthase (iNOS) was increased by inflammatory stimuli in rodents and in asthmatic airways. NO gas had previously been detected in the airways of rodents and a single human breath sample by mass spectrometry. In collaboration with Dr Logan-Sinclair from Medical Engineering in the Royal Brompton Hospital, we were able to develop the first chemiluminescence analyser to detect NO in exhaled air, with a sensitivity down to 1 part per billion (ppb). NO is detectable in normal subjects with levels of around 10ppb, whereas in patients with untreated asthma values of over 25ppb are usually found.

In the initial studies we demonstrated that NO levels in the nasopharynx were extremely high (~1000ppb) and could contaminate measurements of expired NO. We then showed that expiring against an external resistance (~3mm mercury) that the elevation of the soft palate sealed off the nasopharynx and prevented contamination from the upper airways (2). We also demonstrated for the first time that exhaled NO was flow-dependent and that it was very important to regulate expiratory flow. These methods were subsequently adopted by other research groups and formed the basis of the first international guidelines on exhaled NO measurement. We also showed in 1996 that direct sampling from the lower airways through a bronchoscope gave similar values to measurements at the mouth, indicating that exhaled NO reflected local concentrations in the lungs (3).

In 1996 we demonstrated that exhaled NO increased during the late inflammatory response to inhaled allergen in asthmatic patients, whereas it was rapidly reduced in a dose-related manner by inhaled corticosteroids (4) and was virtually abolished by a selective inhibitor of iNOS (5). These early studies, in the 1990s at Imperial, indicated that exhaled NO appeared to reflect the airway inflammation of asthma which increases airway NO via iNOS and is reduced by effective anti-inflammatory therapy. This suggested that exhaled NO could be useful in monitoring asthmatic airway inflammation and therefore therapy. In later studies we demonstrated that exhaled NO returned to normal values in asthmatics treated effectively with inhaled corticosteroids but in

severe asthma patients the levels were often increased, indicating persistent inflammation despite even high doses of steroid therapy, indicating a degree of steroid resistance. We also suggested that exhaled NO could be used to monitor compliance with inhaled corticosteroid therapy. In subsequent studies we demonstrated that exhaled NO was correlated with sputum eosinophils, bronchial eosinophils and airway hyperresponsiveness. We showed that exhaled NO was also increased in asthmatic patients due to occupational exposure.

We also studied exhaled NO in other airway diseases, showing that it was not increased in patients with chronic obstructive pulmonary disease (COPD), except during acute exacerbations and that it may therefore be useful for differentiating from asthma. We showed that exhaled NO was increased in bronchiectasis, but decreased in cystic fibrosis and smokers as NO was converted to soluble nitrate by superoxide anions. We also demonstrated that nasal and exhaled NO are markedly reduced in primary ciliary dyskinesia (6). All of these observations were subsequently confirmed by other research groups.

**3. References to the research** (indicative maximum of six references)

(1) Kharitonov, S.A., Yates, D., Robbins, R.A., Logan-Sinclair, R., Shinebourne, E., Barnes, P.J. (1994). Increased nitric oxide in exhaled air of asthmatic patients. *Lancet*, 343,133-135. DOI. Times cited: 1007 (as at 5th November 2013 on ISI Web of Science). Journal Impact Factor: 39.06.

(2) Kharitonov, S.A., Barnes, P.J. (1997). Nasal contribution to exhaled nitric oxide during exhalation against resistance or during breath holding. *Thorax*, 52, 540-544. DOI. Times cited: 77 (as at 5th November 2013 on ISI Web of Science). Journal Impact Factor: 8.37.

(3) Kharitonov, S.A., Chung, K.F., Evans, D.J., O'Connor, B.J., Barnes, P.J. (1996). Increased exhaled nitric oxide in asthma is mainly derived from the lower respiratory tract. *Am J Respir Crit Care Med*, 153, 1773-1780. DOI. Times cited: 214 (as at 5th November 2013 on ISI Web of Science). Journal Impact Factor: 11.04.

(4) Kharitonov, S.A., Yates, D.H., Barnes, P.J. (1996). Inhaled glucocorticoids decrease nitric oxide in exhaled air of asthmatic patients. *Am J Resp Crit Care Med*, 153, 454-457. DOI. Times cited: 397 (as at 5th November 2013 on ISI Web of Science). Journal Impact Factor: 11.04.

(5) Hansel, T.T., Kharitonov, S.A., Donnelly, L.E., Erin, E.M., Currie, M.G., Moore, W.M. et al. (2003). A selective inhibitor of inducible nitric oxide synthase inhibits exhaled breath nitric oxide in healthy volunteers and asthmatics. *Faseb J*, 17, 1298-1300. DOI. Times cited: 93 (as at 5th November 2013 on ISI Web of Science). Journal Impact Factor: 5.70.

(6) Loukides, S., Kharitonov, S., Wodehouse, T., Cole, P.J., Barnes, P.J. (1998). Effect of arginine on mucociliary function in primary ciliary dyskinesia. *Lancet*, 352, 371-372. DOI. Times cited: 45 (as at 5th November 2013 on ISI Web of Science). Journal Impact Factor: 39.06.

Key funding:
- Asthma UK (National Asthma Campaign) (1994-1996; £120,000), Principal Investigator (PI) P. Barnes and S. Kharitonov, Measurement and significance of exhaled nitric oxide in asthma.
- Asthma UK (2000-2001; £130,000), PI P. Barnes and S. Kharitonov, Peroxynitrite in asthma.
- Novartis (2004-2007; £274,000), PI P. Barnes and S. Kharitonov, Biomarkers in COPD.
- AstraZeneca (2002-2007; £1,150,000), PI P. Barnes and S. Kharitonov, Exhaled biomarkers in asthma.

**4. Details of the impact** (indicative maximum 750 words)

Impacts include: health and welfare, public policy and services, practitioners and services, commerce
Main beneficiaries include: practitioners, patients, British and American Thoracic Societies, medical

device industry

On the basis of research initiated at Imperial measurement of exhaled NO has now become routine in clinical practice and is commonly used for the diagnosis and management of asthma [1] and in clinical trials to assess the effects of novel anti-inflammatory treatments in asthma [2].

International guidelines for the clinical use of exhaled NO by the American Thoracic Society in 2011 recommend the use of exhaled NO as part of clinical practice in asthma [3]. These guidelines specify that exhaled NO be used to diagnose eosinophilic airway inflammation; to determine the likelihood of responsiveness to corticosteroids in patients with chronic respiratory symptoms due to airway inflammation; to diagnose asthma and to monitor airway inflammation in asthma patients [3; page 603]. The 2012 British Thoracic Society guidelines on management of asthma also include the use of exhaled NO as a non-invasive measure of eosinophil count in children and corticosteroid response [4].

Clinical trials have demonstrated that measurement of exhaled NO to monitor asthma control leads to reduction in exacerbations [5], although this has not been confirmed in unselected patients [6]. Exhaled NO has also been used to monitor and improve adherence with corticosteroid therapy in patients with refractory asthma [7].

In the light of our research several analysers for measuring exhaled NO were developed and marketed. Professor Barnes was an advisor to one of the companies called Aerocrine (Stockholm, Sweden) [7], which became the market leader in this field with annual sales of £15m/year. As a key advisor to Aerocrine Professor Barnes was closely involved in the development of a hand-held NO analyser that is much easier to use in clinical practice (NIOX-MINO). This portable exhaled NO device was approved by the FDA in 2008 and has sold over 6 million tests since then [8].

Following our demonstration that nasal NO is very low in patients with primary ciliary dyskinesia (PCD) [9], nasal NO is now recommended in the 2012 European guidelines for the screening for PCD [9].

**5. Sources to corroborate the impact** (indicative maximum of 10 references)

[1] Barnes, P.J., Dweik, R.A., Gelb, A.F., Gibson, P.G., George, S.C., Grasemann, H. et al. (2010).Exhaled nitric oxide in pulmonary diseases: a comprehensive review. *Chest*, 138, 682-692. DOI. [Recent review on the clinical indications for exhaled NO].

[2] Corren, J., Lemanske, R.F., Hanania, N.A., Korenblat, P.E., Parsey, M.V., Arron, J.R. et al. (2011). Lebrikizumab Treatment in Adults with Asthma. *N Engl J Med*, 365, 1088-1098. DOI. [Recent example of a large clinical trial where exhaled NO was an outcome measurement].

[3] American Thoracic Society Clinical Practice Guidelines: Interpretation of exhaled nitric oxide level ($Fe_{NO}$) 2011http://www.atsjournals.org/doi/pdf/10.1164/rccm.9120-11ST. Archived on 5th November 2013.

[4] British Thoracic Society-British Guideline on the Management of Asthma, A National Clinical Guideline 2012 http://www.brit-thoracic.org.uk/guidelines/asthma-guidelines.aspx. Archived on 5th November 2013.

[5] Powell, H., Murphy, V.E., Taylor, D.R., Hensley, M.J., McCaffery, K., Giles, W. et al. (2011). Management of asthma in pregnancy guided by measurement of fraction of exhaled nitric oxide: a double-blind, randomised controlled trial. *Lancet*, 378,983-990. DOI. [Studies showing clinical benefit of exhaled NO monitoring in selected patients]

[6] Szefler, S.J., Mitchell, H., Sorkness, C.A., Gergen, P.J., O'Connor, G.T., Morgan, W.J. et al. (2008). Management of asthma based on exhaled nitric oxide in addition to guideline-based treatment for inner-city adolescents and young adults: a randomised controlled trial. *Lancet*,

372, 1065-1072. DOI. [Study showing no benefit of exhaled NO in unselected patients].

[7] http://www.aerocrine.com/ [Website for Aerocrine, the most successful company marketing analyses for monitoring exhaled NO]. Archived on 26th November 2013.

[8] http://www.medgadget.com/2008/03/fda_approves_niox_mino_for_asthma_monitoring.html [FDA approval of portable exhaled NO monitoring device (NIOX MINO) in 2008]. Archived on 26th November 2013.

[9] Strippoli, M.P., Frischer, T., Barbato, A., Snijders, D., Maurer, E., Lucas, J.S. et al. (2012). Management of primary ciliary dyskinesia in European children: recommendations and clinical practice. *Eur Respir J*, 39 (6), 1482-1491. DOI. [European guidelines for diagnosis and management of PCD, recommending nasal NO as a screening test].