

# EXHALED NITRIC OXIDE: A NEW LUNG FUNCTION TEST

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### Introduction:

Nitric oxide (NO) is produced by many cells within the respiratory tract and endogenous NO may play an important role in the physiological control of airway function and in the pathophysiology of airway diseases<sup>1</sup>. The fraction of exhaled NO (FE<sub>NO</sub>) is a promising biomarker for the diagnosis, follow up and a guide to therapy in adults and children with asthma. The breath test has recently become available in many well -equipped hospitals in developed countries. However, interpretation of exhaled NO measurements may be difficult and there is a wide variation in the reported levels of NO in exhaled air, suggesting that technical factors are important<sup>2</sup>.

### History:

Until the 1980s, nitric oxide, a product of fossil fuel combustion, was thought only to play detrimental effects of air pollution on the respiratory tract. In 1987 experiments with coronary arteries showed that nitric oxide was the long sought endothelium-derived relaxing factor. Once scientists realized that NO played a biological role, its role as a cell signaling molecule and neurotransmitter became clear from abundant studies<sup>3</sup>. NO was first detected in exhaled breath samples in 1991<sup>4</sup>. In 1993, researchers from the Karolinska Institute in Sweden were first to report increased eNO in asthmatics<sup>5</sup>.

### Biology:

Endogenous NO is generated from the amino acid l-arginine by the enzyme NO synthase (NOS) of which three distinct isoform exist: inducible (iNOS), endothelial(eNOS), and neuronal (nNOS)<sup>6</sup>. The latter two are constantly

active in endothelial cells and neurons respectively, whereas iNOS action can be induced in states like inflammation.<sup>1</sup>

It was initially thought that exhaled NO derived mostly from the sinuses, which contain high levels of NO. It has subsequently been shown that the lower airways contribute most of the exhaled NO, and the contamination from the sinuses is minimal<sup>7</sup>.

### Measurement techniques:

The most widely used technique to measure Exhaled nitric oxide is a chemical reaction that produces light; this is called a chemiluminescence reaction. The NO in the breath sample reacts with ozone to form nitrogen dioxide in an excited state. When this returns back to its ground state, it emits light in quantities that are proportional to the amount of exhaled NO. The subject can exhale directly into a measurement device (online technique), or into a reservoir that can afterwards be connected to the analyser (offline technique)<sup>8</sup>. With the former technique, the early and later NO in the breath sample can be analyzed separately. The test requires little coordination from the subject, and children older than 4 can be tested successfully<sup>9</sup>.

### Reference range:

The upper normal level of eNO in different studies ranges from 20 to 30 parts per billion. Men have higher eNO values than women. Smoking notoriously lowers eNO values ,and even former smoking status can influence results .The levels are higher in people with an atopic constitution <sup>10</sup>. The fraction of eNO is also flow - dependent ( higher at lower flow rates and vice versa ) , so measurements are

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normally measured at 50 ml /s . Age or height could also considerably confound eNO values in children. The magnitude of these effects lies in the order of 10%, so even single cut -off values might be useful <sup>11</sup>.

### **Factor affecting exhaled NO in normal individuals:**

Breath holding causes a significant rise in exhaled NO. In normal individuals there is significant increase in NO in exhaled air with respiratory tract infection. This may be a reflection of iNOS induction by virus infection in the upper airways.<sup>12</sup> The effect of exercise is complex, with a progressive fall in exhaled NO with increasing exercise but correction for increased ventilation shows an increase in production of NO. Chronic cigarette smokers have lower levels of exhaled NO than non smokers and there is close correlation between the reduction in exhaled NO and the number of cigarettes smoked<sup>13</sup>.

**Table-I**

*Factors affecting exhaled nitric oxide (NO)<sub>12</sub>*

Increased NO	Decreased NO
Breath holding	Cigarette smoking
Exercise /hyperventilation	Pulmonary hypertension
L-arginine( oral )	Kartageners syndrome
Upper respiratory tract infections	Glucocorticoids
Asthma	NOS inhibitors
Allergen challenge (late response )	
Bronchiectasis	
Cystic fibrosis	
Lower respiratory tract infection	

### **Effect of disease on exhaled NO**

#### ***Asthma***

An increase in exhaled NO in patients with asthma has been reported in several studies<sup>14,15</sup>. This suggests that increased NO is associated with the inflammatory late

response and may be a reflection of iNOS expression in response to inflammatory cytokines. There is also an increase in exhaled NO during exacerbations of asthma <sup>16</sup> and when the maintenance dose of inhaled glucocorticoids is reduced<sup>17</sup>. The increased levels of exhaled NO during an acute exacerbation of asthma are reduced within 48 hours of starting methylprednisolone treatment<sup>16</sup>. All of these findings suggest that exhaled NO may reflect airway inflammation in asthma, and may be used as a means of monitoring inflammatory events in the lower airways.

#### ***Bronchiectasis***

Raised levels of exhaled NO have also been detected in patients with bronchiectasis, and the level of NO is related to the extent of disease as measured by a computed tomography score <sup>18</sup>. This suggests that exhaled NO in bronchiectasis may reflect active inflammation in the lower airways and may be used to monitor disease activity.

#### ***Vascular disease***

In patients with pulmonary hypertension secondary to systemic sclerosis there is a reduction in exhaled NO compared with normal subjects and with patients with interstitial lung disease without pulmonary hypertension<sup>19</sup>. A reduction in exhaled NO has also been reported in systemic hypertension; this is more difficult to explain , but may reflect a generalized defect in endothelial NOS function<sup>20</sup>.

#### **Functional relevance of exhaled NO**

NO gas may be a useful marker of airway and pulmonary disease, but it may also play a physiological and pathophysiological role. The high concentrations of NO generated in the paranasal sinuses may have a sterilizing effect in the sinuses and upper respiratory tract, since NO is toxic to bacteria, parasites and viruses<sup>21</sup>. NO derived from the lower respiratory tract may also contribute to host defense mechanism in the respiratory tract. It is a potent vasodilator and increased production of this in asthmatic airways may cause hyperaemia in the airways of this patients<sup>22</sup>.

**Conclusion:**

The measurement of exhaled NO may provide a simple non-invasive means of measuring airway inflammation. The great advantage of exhaled NO is that the measurement is completely non-invasive and can be performed repeatedly. It is also beneficial for children and patients with severe air flow obstruction where more invasive techniques are not possible. The current available device for exhaled NO measurement are expensive, but it is likely that technological advances will make it possible to miniaturize these analyzers so that they are portable and may even be used at home. This may lead to useful screening measurement for community studies.

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