Exhaled Nitric Oxide: New Opportunities in the Lab

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Disclaimer

• The views in this lecture are those of the presenter and not those of the Department of Veterans Affairs or any other agency of the United States Government.

• I have no vested interests in any of the manufacturers of the equipment referenced in this presentation.
Objectives

- Non-asthmatic pulmonary uses of exhaled nitric oxide monitoring
- Extrapulmonary uses of exhaled nitric oxide monitoring
Abbreviations Used in This Presentation

- FeNO  Fraction of exhaled nitric oxide
- eNO   exhaled nitric oxide
- iNOS  inducible nitric oxide synthase
- HPS   Hepatopulmonary Syndrome
- IPVD  intrapulmonary vascular dilatations
- CBDL  common bile duct ligation
- PVL   portal vein ligation
- RT    radiation therapy
Nitric Oxide

- Potent systemic and pulmonary vasodilator
- Increased levels: increased inflammation
- Decreased levels: role in Pulm HTN?
- Deleting the eNOS gene leads to Pulm HTN (which is reversed by inhaling NO)
Nitric Oxide Synthase

- NOS enzyme in the production of NO
- O2 a necessary substrate
- Deleting the eNOS gene leads to Pulm HTN
Nitric Oxide Synthase

- **Type I**: Neuronal Nitric Oxide Synthase
- **Type II**: Inducible Nitric Oxide Synthase
- **Type III**: Endothelial Nitric Oxide Synthase

- Pulmonary vasculature: mainly Type II, iNOS
- Airways: all three
An Official ATS Clinical Practice Guideline: Interpretation of Exhaled Nitric Oxide Levels (\(F_{ENO}\)) for Clinical Applications

Raed A. Dweik, Peter B. Boggs, Serpil C. Erzurum, Charles G. Irvin, Margaret W. Leigh, Jon O. Lundberg, Anna-Carin Olin, Alan L. Plummer, D. Robin Taylor, on behalf of the American Thoracic Society Committee on Interpretation of Exhaled Nitric Oxide Levels (\(F_{ENO}\)) for Clinical Applications

This Official Clinical Practice Guideline of the American Thoracic Society (ATS) was approved by the ATS Board Of Directors, May 2011

https://www.thoracic.org/statements/resources/allergy-asthma/feno-document.pdf
### TABLE 5. GENERAL OUTLINE FOR FeNO INTERPRETATION: SYMPTOMS REFER TO COUGH AND/OR WHEEZE AND/OR SHORTNESS OF BREATH*

<table>
<thead>
<tr>
<th>FeNO</th>
<th>Symptoms present during past 6+ wk</th>
<th>Diagnosis</th>
<th>Monitoring (in Patients with Diagnosed Asthma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 25 ppb (&lt;20 ppb in children)</td>
<td>Eosinophilic airway inflammation unlikely, Alternative diagnoses, Unlikely to benefit from ICS</td>
<td>Be cautious, Evaluate clinical context, Monitor change in FeNO over time</td>
<td>Persistent allergen exposure, Poor adherence or inhaler technique, Inadequate ICS dose, Risk for exacerbation, Steroid resistance, ICS withdrawal or dose reduction may result in relapse</td>
</tr>
<tr>
<td>25–50 ppb (20–35 ppb in children)</td>
<td></td>
<td></td>
<td>Persistent allergen exposure, Poor adherence or inhaler technique, Inadequate ICS dose, Risk for exacerbation, Steroid resistance, ICS withdrawal or dose reduction may result in relapse</td>
</tr>
<tr>
<td>&gt; 50 ppb (&gt;35 ppb in children)</td>
<td></td>
<td></td>
<td>Persistent allergen exposure, Poor adherence or inhaler technique, Inadequate ICS dose, Risk for exacerbation, Steroid resistance, ICS withdrawal or dose reduction may result in relapse</td>
</tr>
</tbody>
</table>

**Definition of abbreviations:** FeNO = fraction of exhaled nitric oxide; ICS = inhaled corticosteroid.

* The interpretation of FeNO is an adjunct measure to history, physical exam, and lung function assessment. See text and Tables 3 and 4 for other details.

[www.thoracic.org/statements/resources/allergy-asthma/feno-document.pdf](http://www.thoracic.org/statements/resources/allergy-asthma/feno-document.pdf)
<table>
<thead>
<tr>
<th>LOW FeNO LEVEL</th>
<th>INTERMEDIATE/INCREASING FeNO LEVEL*</th>
<th>HIGH FeNO LEVEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;25 ppb in ≥12 years of age</td>
<td>25-50 ppb in ≥12 years of age</td>
<td>&gt;50 ppb in ≥12 years of age</td>
</tr>
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<td>&lt;20 ppb in &lt;12 years of age</td>
<td>20-35 ppb in &lt;12 years of age</td>
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- Eosinophilic inflammation less likely

- Symptomatic† patients unlikely to benefit from ICS therapy; consider other possible etiologies‡

- Cautious interpretation; based on clinical judgment, consider initiating ICS therapy and monitor change in FeNO levels

- Symptomatic patients likely to benefit from ICS therapy; investigate allergen exposure

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Lung Transplant

• Monitored serial FeNO in lung transplant recipients
• Gashouta, MA et al
• J Heart Lung Transplant. 2015 Apr;34(4):557-62
Lung Transplant

• Exhaled Nitric Oxide in Human Lung Transplantation: A Noninvasive Marker of Acute Rejection

• Silkoff, PE et al
Lung Transplant

- Usefulness of exhaled nitric oxide to guide risk stratification for bronchiolitis obliterans syndrome after lung transplantation.
- Neurohr C et al
Lung Transplant

• Non-invasive assessment of exhaled biomarkers in lung transplantation
• Yates, DH et al
• J Breath Res 2011 Jun; 5(2)
Lung Transplantation

- Exhaled nitric oxide after lung transplantation: impact of the native lung
- Verleden et al
Lung Transplant

• Exhaled nitric oxide and carbon monoxide in lung transplanted patients
• Cameli, P et al
Hepatopulmonary Syndrome (HPS)

- Severe liver disease of varied etiologies
- Diagnostic Triad
  - Liver disease
  - Impaired oxygenation
  - Intrapulmonary vascular abnormalities: intrapulmonary vascular dilatations (IPVDs)
- Pulmonary A-V shunts and dilation of precapillary pulmonary arteries
Liver Diseases in HPS

- Many chronic and severe
- But acute hepatic disease such as ischemic hepatitis
- Approximately 80% patients present with symptoms consistent with liver disease
- The remaining commonly present with dyspnea
Dyspnea

• Dyspnea in HPS often associated with the following:
  – Dyspnea on exertion, rest or both
  – Orthodeoxia: decrease in PaO2 of > 4 mmHg when going from the supine to upright position
  – Platypnea: worsening of dyspnea when going from the supine to upright position
Hypoxemia

• *Causes of hypoxemia in liver disease can be due to*
  – Atelectasis: mild hypoxemia
    • Compression of lung parenchyma by ascities, pleural fluid
  – Portal HTN (c/s cirrhosis): mild hypoxemia
  – HPS: severe hypoxemia
Etiology of IPVDs

• Impaired hepatic function cannot clear circulating pulmonary vasodilators
• Production of circulating vasodilators by impaired liver
• Inhibition of vasoconstrictive substances with hepatic impairment
Hypothesis: Do Pts with HPS have \( \uparrow \) eNO?

- Cremona et al studied 12 healthy subjects, 6 pts with cirrhosis, 3 with cirrhosis and severe hypoxemia and orthodeoxia.
- All 9 cirrhosis patients awaiting transplant
  - Chronic active hepatitis (2)
  - Cryptogenic cirrhosis (5)
  - Primary biliary cirrhosis (1)
  - Primary sclerosis cholangitis (1)
- No one in study had evidence of airway obstruction

Cremona et al Eur Resp J 1995, 8, 1883-1885
Hypothesis: Do Pts with HPS have ↑ eNO?

- Technology of the day did not allow single breath eNO measurement
- 10 minutes breathing NO-free air from a 100 L Douglas bag (<1 ppb NO)
- Exhaled into a second 100 L Douglas bag
- Normal subjects also inhaled hypoxic gas mixture of 10% oxygen
Hypothesis: Do Pts with HPS have \( \uparrow \) eNO?

- All test subjects had CXR, Spirometry and DLCO, Pulsoximetry
- All subjects had nml CXR and spirometry

| Table 1. – Lung function, arterial blood gases and rate of production of nitric oxide |
|-------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                               | Hepatopulmonary patients | Cirrhotic controls | Normal controls |
|                               | No. 1 Breathing air | No. 2 Breathing air | No. 3 Breathing air | (n=6) Breathing air | (n=18) Breathing air | (n=12) Breathing 10% O_2 |
| \( T_L,CO \) mmol·kPa\(^{-1}\)·min\(^{-1} \) | 5.4              | 4.93            | 3.35            | 6.71±0.3         | 8.6±0.5          | – |
| \( P_{a,O_2} \) kPa            | 8.5              | 6.6             | 8.2             | 13.2±1.6         | 96–100           | 70–87 |
| \( S_{a,O_2} \) %              | 93               | 85              | 91              | 95–97            | –               | – |
| Shunt fraction %               | 21               | 26              | 17              | 3.4±1.8          | 3.3±1.8          | 2.8±1.6 |
| MNO nM·min\(^{-1} \)           | 9.2              | 8.7             | 12.5            | 3.4±1.8          | –               | – |
| MNO after transplantation nM·min\(^{-1} \) | 4.7              | –               | –               | –               | –               | – |

\( T_L,CO \): transfer factor of the lungs for carbon monoxide; \( P_{a,O_2} \): arterial oxygen tension; \( S_{a,O_2} \): arterial oxygen saturation; MNO: Molar rate of production of nitric oxide.
Table 1. - Lung function, arterial blood gases and rate of production of nitric oxide

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<td></td>
<td>No. 1 Breathing air</td>
<td>No. 2 Breathing air</td>
<td>No. 3 Breathing air</td>
</tr>
<tr>
<td>TL,co mmol·kPa⁻¹·min⁻¹</td>
<td>5.4</td>
<td>4.93</td>
<td>3.35</td>
</tr>
<tr>
<td>PₐO₂ kPa</td>
<td>8.5</td>
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<td>8.2</td>
</tr>
<tr>
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TL,co: transfer factor of the lungs for carbon monoxide; PₐO₂: arterial oxygen tension; SₐO₂: arterial oxygen saturation; MNO: Molar rate of production of nitric oxide.
Hypothesis: Do Pts with HPS have ↑ eNO?

- Normal subjects breathing the hypoxic gas mixture did not have an increase in eNO
- One cirrhotic patient underwent lung transplant during the study
- This subject's eNO decreased after transplant
- Does the transplant correct the V/Q mismatch and IPVDs
Enhanced Endothelin B receptor expression, Endothelin-1 mediated eNOS and NO production in HPS

• Luo, in animal studies, noted that CBDL increased pulmonary endothelial nitric oxide synthase levels
  – intrapulmonary vasodilatation
  – gas exchange abnormalities
  – analogous with HPS
  – these findings found in pulmonary artery samples, not aortic samples
Vascular NO a Component of eNO

• Cremona demonstrated that NO produced by vascular endothelium is a component of eNO

• Pearl found that hypoxemia decreased levels of NO

• Both aortic and pulmonary arterial samples tested
  – Pearl et al J Thorac Cardiovasc Surg 2000, 119, 931-938
Fig. 4. ET-1 stimulation of eNOS protein levels in PA and AO segments isolated from normal, PVL and CBDL animals. Segments were treated with 0.1 nM ET-1 in the presence or absence of a selective $\text{ET}_A$ (20 $\mu$M TBC3214Na) or a selective $\text{ET}_B$ (15 $\mu$M BQ-788) receptor antagonist for 24 h. Top panel summarizes ET-1 effects on eNOS protein levels in PA segments from normal, PVL and CBDL animals. Bottom panel summarizes ET-1 effects on eNOS protein levels in AO segments from normal, PVL and CBDL animals. Data are expressed as mean ± SE fold untreated normal, PVL or CBDL as control values ($n = 4$–$5$ for each group). *$P < 0.05$ compared with untreated control.
Both O2 and NO compete for binding on hemoglobin (on same heme iron sites) NO binds faster than CO or O2
Exhaled NO and Oxygenation Pre /Post Liver Transplantation in Cirrhosis

• Rolla studied 20 patients with cirrhosis and no history/risk factors for cardiopulmonary disease before and after liver transplant

• After transplant
  – exhaled NO decreased
  – A-a DO2 decreased
  – 5 Pts with HPS- no longer evident as demonstrated by contrast-enhanced echocardiography prior to surgery

# Table

Arterial Blood Gas Analyses, Exhaled Nitric Oxide Concentrations, and Patients with the Hepatopulmonary Syndrome before and after Liver Transplantation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before Transplantation</th>
<th>After Transplantation</th>
<th>Difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{Pao}_{2}, \text{ mm Hg} )</td>
<td>86.8 ± 9.9</td>
<td>90.1 ± 6.7</td>
<td>-3.3 (-7.8 to 1.3)</td>
</tr>
<tr>
<td>( \text{Paco}_{2}, \text{ mm Hg} )</td>
<td>32.7 ± 4.2</td>
<td>37.5 ± 2.7</td>
<td>-4.8 (-7.1 to -2.5)</td>
</tr>
<tr>
<td>Alveolar–arterial oxygen gradient, mm Hg</td>
<td>17.3 ± 7.1</td>
<td>9.0 ± 5.2</td>
<td>8.3 (5.2 to 11.4)</td>
</tr>
<tr>
<td>Exhaled nitric oxide concentration, ppb</td>
<td>13 ± 4.9</td>
<td>6.2 ± 2.8</td>
<td>6.8 (4.7 to 8.9)</td>
</tr>
<tr>
<td>Patients with hepatopulmonary syndrome, n†</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* ppb = parts per billion.
† All values except those in the last row are the mean ± SD.
‡ Hepatopulmonary syndrome defined by alveolar–arterial oxygen gradient > 15 mm Hg and positive results on contrast-enhanced echocardiography. \( P < 0.005 \) (McNemar test for paired observation) for difference before and after transplantation.
Exhaled NO and Oxygenation Pre /Post Liver Transplantation in Cirrhosis

• Impaired oxygenation in cirrhosis is often corrected with transplantation
• Decrease in exhaled nitric oxide concentrations correlates with improved oxygenation
• Role of nitric oxide in cirrhosis
• Role of nitric oxide in identifying patients at risk of developing HPS
Predictive Value of eNO in Radiation Pneumonitis

- Radiation pneumonitis is a dose-related toxicity of radiation therapy for
  - lung cancer
  - breast cancer
  - mesothelioma
  - esophageal cancer
  - or any radiation treatment of the thorax

- Inflammatory response in normal lung tissue in response to radiation injury

Guerrero; Int J Radiation Oncology Biol Phys 2012 981-988
Predictive Value of eNO in Radiation Pneumonitis

• Symptoms can occur up to 6 months after treatment ends
  – Cough
  – Dyspnea
  – Fever
  – Changes in pulmonary function
Predictive Value of eNO in Radiation Pneumonitis

• Guerrero reviewed 28 patients undergoing RT for esophageal cancer
• eNO measured
  – prior to therapy
  – at completion of last RT session
  – 6 weeks after the last RT session
Predictive Value of eNO in Radiation Pneumonitis

- eNO was elevated in all patients after RT who subsequently developed radiation pneumonitis
- Dose-related
- eNO was elevated weeks to months PRIOR to symptoms appeared
- Koizumie had similar findings in lung cancer patients undergoing RT
FeNO and Cystic Fibrosis

- Grasemann et al
- Demonstrated lower FeNO levels in CF
- Propose elevated arginase activity
- Arginase competes with L-arginine in the NO synthase cycle
FeNO and Bronchiectasis

• Kharitonov et al 1995 AJRCCM
• Demonstrated elevated FeNO levels in bronchiectasis
• FeNO levels correlated with abnormal CT findings
FeNO and Scleroderma

• FeNO studies in Scleroderma are conflicting
• Tiev et al found FeNO levels noted to be elevated in Scleroderma patients with ILD compared to Scleroderma w/o ILD
• Malerba et al found no difference in FeNO levels regardless of lung involvement
Pulmonary HTN

- Fractional exhaled nitric oxide in pulmonary hypertension
- El Chami, H et al
- Eur Resp J 2016 48: PA2492
FeNO and URIs

• Kharitonov et al reported increased FeNO levels in patients with URIs and no history of lung disease.
• These levels decreased markedly when the same patients were tested three weeks after first measurement and resolution of the URI.
Conditions Associated with Decreased FeNO Levels

- Pulmonary HTN
- Hypothermia
- Primary ciliary dyskinesia
- Bronchopulmonary dysplasia
- Alcohol, tobacco, caffeine...
Elevated eNO Hepatic Disease vs Asthma

• Delclaux et al demonstrated elevated ALVEOLAR eNO levels in patients with cirrhosis and elevated BRONCHIAL eNO levels in patients

• Linear relationship with eNO and expiratory flow

• FeNO$_{50}$ and FeNO$_{200}$ (ml/sec)

• EVA4000 chemiluminescent analyzer used
Omalizumab (Anti-IgE) use in Asthma-COPD Overlap Syndrome (ACOS)

• Yalcin et al Immunopharmacol Immunotoxicol 2016 Apr 28:1-4 (Epub ahead of print)

• After one year of treatment: IgE, FeNO, eosinophils, ..among other biomarkers for inflammation were decreased

• Pts had improved symptoms
References

References

• Delclaux C, Mahut B et al, Increased nitric output from alveolar origin during liver cirrhosis versus bronchial source during asthma. Am J Respir Crit care Med 165: 332-337, 2002
References

References

• Cameli, P et al. Exhaled nitric oxide and carbon monoxide in lung transplanted patients. Respir Med. 2015 Sep; 1224-9
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