Exhaled nitric oxide levels in exacerbations of asthma, chronic obstructive pulmonary disease and pneumonia

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ABSTRACT

Objective: Nitric oxide is known to be present in the exhaled air of normal subjects and at higher concentrations in asthmatics. The aim of this study was to measure exhaled nitric oxide levels in patients admitted to hospital with acute exacerbations of asthma, or chronic obstructive pulmonary disease, or with pneumonia.

Methods: Within 24 hours of admission exhaled nitric oxide levels were measured by a chemiluminescent analyzer in 11 patients with acute sever asthma, 19 patients with acute exacerbation of chronic obstructive pulmonary disease, and in 12 patients with pneumonia. In asthmatics measurements were made on 3 occasions, at day 1, 4, and 28 and were related to changes in peak expiratory flow rate.

Results: On admission median exhaled nitric oxide levels (range) were significantly higher in asthmatics 22 (9.3-74) parts per billion in comparison to patients with chronic obstructive pulmonary disease 10.3 (2.7-34) parts per billion; $p<0.01$, pneumonia 7 (4-17) parts per billion; $p<0.001$, and normal subjects 8.7 (5-13.3) parts per billion; $p<0.001$. Following treatment the asthmatics had a significant reduction in their exhaled nitric oxide levels from 22 (9.3-74) parts per billion on day 1 to 9.7 (5.7-18.3) parts per billion on day 28; $p=0.005$. Peak expiratory flow rate measurements increased from 200 (120-280) l/min on day 1 to 280 (150-475) l/min on day 4; $p<0.05$ and to 390 (150-530) l/min on day 28; $p<0.01$. A strong negative correlation existed between peak expiratory flow rate measurements and exhaled nitric oxide levels in asthmatics on day 28 ($r=-0.70$; $p=0.017$).

Conclusion: Acute exacerbations of asthma are associated with increased levels of exhaled nitric oxide in contrast to exacerbations of chronic obstructive pulmonary disease and acute pneumonia. Exhaled nitric oxide may be a useful indirect marker of asthmatic airway inflammation. The differing time course of response of nitric oxide to peak flow measures suggests that these two measures are reflecting differing airway events.

Keywords: Nitric oxide, asthma, chronic obstructive pulmonary disease, pneumonia.

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Nitric oxide (NO) is produced endogenously from the semi-essential aminoacid L-arginine through the action of the enzyme nitric oxide synthase (NOS), of which at least three isoforms are known, two being constitutive and one inducible.[1] The constitutive isoforms (cNOS) include the endothelial (eNOS) and the neuronal (nNOS) types, which are basically expressed in endothelial and neuronal cells, and produce small amounts of NO on activation. Whereas the inducible isoform (iNOS) is upregulated by pro-inflammatory cytokines,[2] and produces relatively larger amounts of NO. The induction of NOS is regulated by transcription factors, of which nuclear factor-kB is the most important.[3] Asthmatic patients have significantly higher levels of exhaled NO,[4-6] which are probably a reflection of the

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Table 2 - Characteristics of the asthmatics and the results of their exhaled NO levels and peak expiratory flow rates.

				Exhaled NO levels (ppb)+			Peak expiratory flow rate (l/min)			
Patient history	**Age years**	**Sex**	**Smoking history***	**Day 1**	**Day 4**	**Day 28**	**Day 1**	**Day 4**	**Day 28**	**Steroid therapy prior to admission~**
1.	18	F	N	40.7	19	9.7	250	280	450	
2.	27	F	N	16.7	16.3	11	200	320	370	
3.	47	F	S	18.3	8	5.7	130	220	415	
4.	66	F	Ex.17 years	49.7	60.3	16	120	150	300	
5.	65	M	S	74	34.3	18.3	180	220	290	
6.	28	F	Ex. 4 years	22.3	14	13.7	280	310	390	Prednisolone 40mg daily for 5 days
7.	25	M	Ex. 1 month	33	15	8.3	230	475	515	
8.	49	M	Ex. 3 years	29.3	52.7	8	200	150	390	Prednisolone 30mg daily for 7 days
9.	50	M	N	19.7	15	17	150	310	150	Steroid dependent pred. 15mg daily
10.	32	F	Ex. 9 years	9.3	4.7	6	270	430	530	
11.	46	F	N		7.3	9.3	120	190	210	Steroid dependent pred. 30mg daily
Median	41			22	15	9.7	200	280	390	
Range	18-66			9.3-74	4.7-60.3	5.7-18.3	120-280	150-475	150-530	

*Smoking history: S-current smoker, N-life long non-smoker, Ex.-ex-smoker with the time since quitted.
+Exhaled nitric oxide (NO) levels in parts per billion (ppb).
~Oral steroids that patients were taking either regularly or for the current exacerbation.

Table 1 - Characteristics of COPD and pneumonia patients with their exhaled NO levels.

COPD*					**Pneumonia**				
Patient No.	**Age years**	**Sex**	**NO ppb+**	**Smoking ~ history**	**Patient No.**	**Age Years**	**Sex**	**NO ppb+**	**Smoking~ history**
1.	73	M	7.7	S	1.	30	M	14.7	N
2.	67	F	2.7	S	2.	21	M	4	N
3.	65	M	6	S	3.	44	M	7.7	N
4.	361	F	29.7	S	4.	42	M	6.3	N
5.	64	M	11.7	S	5.	63	M	8.7	N
6.	70	M	9	S	6.	41	F	7	N
7.	70	M	12	S	7.	52	F	12	N
8.	76	M	20.7	S	8.	41	M	5.7	N
9.	67	M	4.3	S	9.	73	M	17	Ex. 8 years
10.	76	M	11	Ex. 4 years	10.	70	F	7.3	Ex. 9 months
11.	75	M	5.3	Ex. 6 months	11.	67	M	5	Ex. 2 years
12.	72	M	24.3	Ex 17 years	12.	78	M	9.3	Ex. 20 years
13.	75	F	17.3	Ex. 4 years					
14.	74	F	8.7	Ex. 5 years					
15.	72	M	34.3	Ex 7 years					
16.	70	M	8.3	Ex. 7 years					
17.	68	F	5.7	Ex. 5 years					
18.	87	M	10.3	Ex. 10 years					
19.	68	M	13	Ex. 8 years					
Median	71		10.3			52			
range	61-87		2.7-34.3			21-78			

*COPD: Chronic Obstructive Pulmonary Disease, M-male, F-female,
+Exhaled nitric oxide levels in parts per billion (ppb)
~Smoking history: S-current smoker, N-Life long non-smoker, Ex.-ex smoker with time since quitted.

underlying upregulation of iNOS expression in their airways,[7] and both iNOS expression[8] and exhaled NO levels[6] in patients with mild asthma are reduced by corticosteroid therapy. Acute asthma attacks have been reported to be associated with much higher levels of exhaled NO[9] and in this one study corticosteroid therapy produced improvement both in airway obstruction and exhaled NO levels, suggesting that exhaled NO might be a useful index of both asthma severity and treatment efficacy. Cigarette smoking is associated with several adverse health problems, including an increased risk of chronic obstructive pulmonary disease (COPD)[10] and respiratory tract infections.[11] Cigarette smokers are known to have lower levels of exhaled NO,[12] but reports on exhaled NO levels in acute exacerbations of COPD, as well as pneumonia are lacking. Using a specially designed portable machine (expiratory sampler) for the collection of exhaled air samples for NO analysis we have measured the levels of exhaled NO in patients admitted to hospital for an exacerbation of COPD, pneumonia, or acute asthma using chemiluminescent analyzer. Furthermore, in asthmatics the response to treatment was monitored by serial measurements of peak expiratory flow rate (PEFR) and exhaled NO levels. The study was approved by the Southampton Hospital and University Joint Ethical Committee, and a written informed consent was given by each patient.

Methods. ***Patients.*** We studied three groups of patients, all were admitted to Southampton General Hospital for either an acute exacerbation of asthma, an exacerbation of COPD, or acute pneumonia. For asthma and COPD patients a medical record review confirmed their diagnoses, as defined by the American Thoracic Society,[13] and an acute exacerbation was defined as subjective worsening of their symptoms, inspite of using their regular medications, that requires hospitalization. Pneumonia was defined as an acute respiratory illness with radiographic pulmonary shadowing which was at least segmental and was neither pre-existing nor of other known cause. A total of 19 patients with COPD exacerbations, 12 patients with pneumonia (Table 1), and 11 patients with acute asthma (Table 2) were included in the study. Patients were treated by their admitting physicians with all asthmatics receiving systemic steroids (40-60mg prednisolone daily) and nebulized bronchodilators.

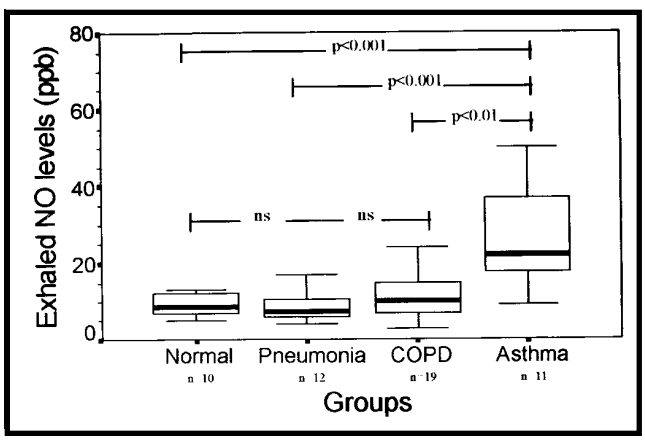

Figure 1 - Box Whisker plot of exhaled nitric oxide (NO) levels, measured in parts per billion (ppb), on day 1 in the three study populations and in normal control subjects. n=number of patients in each group, COPD-chronic obstructive pulmonary disease.

Expiratory sampler. A small portable machine was designed to collect exhaled air samples from acutely ill patients. For collection, air was exhaled into an "empty" chamber, which had been evacuated under suction prior to sampling to ensure no dilution or mixing of the sample with either the ambient air or the residue of the previous sample. The parts and components of the flow path were manufactured from Stainless Steel and the sampler bag was made from PTFE which do not react with oxides of nitrogen, and all parts were housed in a portable box. Control sampling from a known standard have identified a 99.7±0.02% retention of NO during periods of transportation.

Nitric oxide measurements. Exhaled air samples were collected from all patients within 24 hours of admission using the expiratory sampler. While sitting in bed and wearing nose clips patients inhaled and then slowly exhaled into the mouth piece of the portable machine to fill the sampler bag (500ml), to obtain a mixed expired air sample. The expiratory sampler was then taken to the laboratory and connected to the NO analyzer for NO measurements within 5 minutes of sample collection. The plateau concentration in parts per billion (ppb) was recorded and the mean of three separate measurements used for analysis. In asthmatics two further measurements were made on day 4 and day 28 after admission.

Lung function. In asthmatics PEFR were measured using Wright mini peak flow meter (Wright, Derby, UK), immediately before NO measurements and at least 4 hours after bronchodilator inhalation. The best of three readings was considered for evaluation.

Statistical analysis. Statistical analyses were performed using SPSS package (version 6.1). All data are expressed as median (range) unless stated otherwise. Repeated measurement for the same group of patients were analyzed using Friedman's two-way ANOVA, whereas comparisons between different groups of patients were made using Kruskal-Wallis one-way ANOVA. Differences between paired data were analyzed using Wilcoxon matched-pairs signed-ranks test and for unpaired data

using Mann-Whitney U test. Correlations were investigated using Spearman's correlation coefficient. Significance was defined as a p value of <0.05.

Results. ***Exhaled nitric oxide levels.*** Exhaled NO was detected in all patients, and on the day of admission there was a significant difference in the exhaled NO levels between the 4 study populations (ANOVA, p<0.001), with patients with acute asthma having significantly higher median (range) levels of NO at 22 (9.3-74) ppb in comparison to patients with exacerbations of COPD [10.3 (2.7-34)] ppb p<0.01, patients with acute pneumonia [7 (4-17)] ppb p<0.001, or normal subjects [8.7 (5-13.3)] ppb; p<0.001 (Figure 1). There was no significant difference between the exhaled NO levels in COPD, pneumonia patients and normal subjects nor between the COPD patients who were current smokers 9 (2.7-29.7) ppb or ex-smokers 10.7 (5.3-34.3) ppb; p=0.51. Following treatment, there was a significant difference between the repeated measurements of exhaled NO levels in asthmatics (ANOVA; p<0.01), with the levels falling from 22 (9.3-74) ppb on day 1 to 15 (4.7-60.3) by day 4 (ns; p=0.1) and to 9.7 (5.7-18.3) ppb (p=0.005) on day 28.

Peak expiratory flow rate measurements. In the asthmatics the PEFR measurements improved significantly after treatment (ANOVA; p<0.001) and increased from 200 (120-280) l/min on day 1 to 280 (150-475) l/min; p<0.05 on day 4, and further to 390 (150-530) l/min; p<0.01 on day 28. There was a strong negative correlation between exhaled NO levels and PEFR measurements on day 28 (r=-0.70; p=0.017). This was not evident on either day 1 or day 4 of admission.

Discussion. In this study we have demonstrated that patients admitted to hospital with an acute exacerbation of asthma have significantly higher levels of NO in exhaled air on the day of admission than patients with acute exacerbations of COPD, acute pneumonia, or normal subjects (Figure 1). Following treatment with inhaled **b**2-adrenoceptor agonists and systemic corticosteroids there was an increase in PEFR and a fall in exhaled NO levels in those patients with asthma. A significant improvement in pulmonary function was evident earlier during their recovery than a reduction in NO, identifying that change in NO is not purely a reflection of change in airway caliber and that this measure provides additional information about another facet of asthma. Indirect evidence would suggest that NO measure is a reflection of the underlying airway inflammation. Nitric oxide is generated from the semi-essential aminoacid L-arginine by nitric oxide synthase.[1] Increased expression of the inducible form of NOS has been demonstrated within the airways in asthma[7] and shown to be reduced by oral or inhaled corticosteroid therapy.[8] All our patients had been maintained on inhaled corticosteroid therapy prior to their admission and 4 of the 11 patients had been commenced on oral steroids before their admission. Despite this intervention exhaled NO levels were still raised above those levels in the patients with COPD and pneumonia. The severity of their exacerbations may thus explain the slower response to intervention in NO levels in exhaled air than in the one previously published report of NO levels in acute exacerbations of asthma. In the study by Massaro and co-workers[9] exhaled NO levels returned to normality by 48 hours of intervention but only 2 of their 5 patients studied had received inhaled corticosteroids prior to admission and none had received oral steroids. In this previous study it was not possible to ascertain the specificity of the findings for asthma nor to exclude a contribution for the reduction in NO to bronchodilatation. By studying patients with acute pneumonia and exacerbations of COPD in our study comparison has been made with two other forms of acute pulmonary diseases. Neither of these illnesses were associated with elevations in exhaled NO levels above that identified in normal subjects using collected expired air samples[14,15] or above reported levels in COPD patients when in a stable condition.

Both COPD[16] and pneumonia are associated with neutrophilic inflammation whilst asthma is an eosinophilic airway disease.[17] These findings thus suggest a specificity for elevations in exhaled NO in eosinophilic rather than neutrophilic lung inflammation. Studies on changing the dose of prophylactic anti-inflammatory therapy in asthma[18] have suggested that changes in exhaled NO occur before other forms of monitoring, such as peak flow, making NO a more sensitive index of changes within the airways. In our study peak flows improved before changes in exhaled NO during the recovery phase from acute asthma. While this might indicate that peak flow is a more sensitive measure of improvement it could also indicate that NO is a more valuable marker of the underlying inflammatory airway process, being uninfluenced by changes in airway tone[19] whereas peak flow although indirectly reflecting underlying airway pathology will also be acutely influenced by bronchodilator therapy. Thus NO may be a reliable indicator of airway events in patients receiving long acting b2-adrenoceptor agonists in whom peak flow will not be a reliable indicator of underlying airway inflammatory events.

Although NO is being used under these circumstances as an indirect marker of underlying airway events in asthma, NO is likely itself be contributing to disease pathogenesis. NO is known to be a mild bronchodilator in asthma[20] as well as a

vasodilator[21] and as a potential inducer of cell dysfunction, in particular of epithelial cell damage in asthma due to its location of synthesis and its ability to interact with superoxide anions with the formation of potentially harmful peroxynitrites.[22] We were unable to identify any relationship between exhaled NO levels and levels of peak flow on admission but on day 28, once oral steroid therapy had been discontinued as had nebulized **b**2-agonist therapy, and the acute changes known to occur in acute asthma had resolved, there was a significant inverse relationship between exhaled NO and peak flow. Those patients with the highest levels of NO at this stage also having the lowest peak flow. While correlations are associations rather than implying causality, these findings would be consistent with NO in part contributing to asthma severity or reflective of the underlying processes associated with severity of airflow obstruction. Both iNOS[7] and endothelin[23] are expressed in the airway epithelium in asthma, being regulated by the transcription factor NF-kB, and these findings may reflect the co-expression of endothelin, as this peptide is a potent bronchoconstrictor and elevated levels of endothelin have been reported in bronchoalveolar lavage with acute asthma[24] and endothelin levels decrease with corticosteroid therapy,[25] although not completely so in all patients. Further direct investigations within the airways will allow the better understanding of the interrelationship between iNOS and other inflammatory events and the relationship of NO to these processes but the present findings provide further support for exhaled NO mesurement as an indirect marker of airways disease in asthma.

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