

# Gaseous nitric oxide to treat antibiotic resistant bacterial and fungal lung infections in patients with cystic fibrosis: a phase I clinical study

Caroline Deppisch<sup>1</sup> · Gloria Herrmann<sup>1</sup> · Ute Graepler-Mainka<sup>1</sup> · Hubertus Wirtz<sup>2</sup> · Susanne Heyder<sup>3</sup> · Corinna Engel<sup>1</sup> · Matthias Marschal<sup>5</sup> · Christopher C. Miller<sup>4</sup> · Joachim Riethmüller<sup>1,6</sup>

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## Abstract

**Background** Individuals with cystic fibrosis (CF) receive antibiotics continuously throughout their entire life which leads to drug resistant microbial lung infections which are difficult to treat. Nitric oxide (NO) gas possesses antimicrobial activity against a wide variety of microorganisms in vitro, in vivo in animal models and a phase I study in healthy adults showed administration of intermittent 160 ppm NO to be safe.

**Methods** We assessed feasibility and safety of inhaled NO in eight CF patients who received 160 ppm NO for 30 min, three times daily for 2 periods of 5 days.

**Results** The NO treatment was safe and in none of the patients were serious drug-related adverse events observed which caused termination of the study. The intention-to-treat analysis revealed a significant mean reduction of the colony forming units of all bacteria and all fungi, while

mean forced expiratory volume 1 s % predicted (FEV<sub>1</sub>) relative to baseline increased  $17.3 \pm 8.9$  % ( $P = 0.012$ ).

**Conclusions** NO treatment may improve the therapy of chronic microbial lung infections in CF patients, particularly concerning pathogens with intrinsic or acquired resistance to antibiotics.

**Keywords** Cystic fibrosis · Lung infection · Antimicrobial · Nitric oxide

## Introduction

The genetic disorder cystic fibrosis (CF) caused by mutations in the gene CF Transmembrane Conductance Regulator (CFTR) [1–3] affects approximately 80,000 Caucasian individuals in Europe and North America. In airways of CF patients, mutations in the epithelial chloride channel *CFTR* provoke chronic microbial infection and inflammation, leading to excessive lung tissue destruction which mostly determine morbidity and mortality of the patients [4].

Antibiotic therapy shortly initiated after colonization of the airways by *P. aeruginosa*, the major pathogen in CF, has led to high eradication rates [5]. However, once *P. aeruginosa* lung infections become chronic, antibiotics even at high doses do not eradicate the pathogens in the CF airways due to bacterial biofilm formation [6], development of mutator strains [7], and drug adsorption to sputum components [8]. Besides the triad *P. aeruginosa*, *Staphylococcus aureus* and *Haemophilus influenzae*, a huge microbiota is present in CF airways, comprising more than 150 bacterial families which may contribute to the complex pathophysiology of lung disease in CF [5, 9].

In CF patients who receive the highest cumulative antimicrobial drug load in their life time, the worldwide

D. Caroline and H. Gloria shared first authorship.

✉ Joachim Riethmüller  
joachim.riethmueller@med.uni-tuebingen.de

- 1 Comprehensive Cystic Fibrosis Center, Children's Clinic, Tübingen, Germany
- 2 Medizinische Klinik I, Pneumologie, Universität Leipzig, Leipzig, Germany
- 3 Klinik Schillerhöhe, Robert Bosch Krankenhaus GmbH, Gerlingen, Germany
- 4 Nitric Oxide Laboratory, Division of Respiratory and Infectious Diseases, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada
- 5 Institute of Medical Microbiology and Hygiene, Universitätsklinikum Tübingen, Tübingen, Germany
- 6 University Children's Hospital, Universitätsklinikum Tübingen, Hoppe-Seyler-Str. 1, 72076 Tübingen, Germany

increasing proportions of antibiotic resistant strains is particularly evident [10, 11]. In 2001, methicillin-resistant *S. aureus* (MRSA) was found to grow from respiratory tract secretions of only 7 % of CF patients, compared with 25.7 % of patients in 2010 [12, 13]. Furthermore, there is alarming evidence of increasing transmissible epidemic strains that prevail in major European centers [14, 15]. Importantly, some of these pathogens such as *Achromobacter xylosoxidans*, *Burkholderia complex species*, non-tuberculous mycobacteria, and *Aspergillus* species display intrinsic resistances to currently available antibiotics which makes therapy of lung disease in CF additionally difficult. Taken together, there is an urgent need to develop alternative treatment strategies.

Nitric oxide (NO) is a hydrophobic, free-radical, nano-molecular gas that is naturally produced in the body and has a major role in innate immunity. NO exhibits broad reactivity and rapid diffusive properties through biological liquids and lipid membranes and NO has a short half-life (seconds) in a physiological milieu [16, 17]. Utilizing a variety of exogenous NO vehicles, it has been shown that NO possesses antimicrobial activity in vitro against a wide variety bacteria, viruses, fungi, helminthes, and parasites [18–22]. Miller et al. [23] identified the lowest effective antimicrobial inhaled NO gas dose to be approximately 160 parts per million (ppm) hypothesizing the potential for NO as an inhalational antimicrobial therapy. Despite the challenges required to administer NO safely, an effective and safe delivery regimen in animal models has since been validated [25, 26]. Recently, their group demonstrated in healthy adults that multiple 30 min doses of 160 ppm appeared to be safe and well tolerated (safety) [27].

Here we report on the feasibility and safety of inhaling 160 ppm gaseous NO in eight CF patients. Intermittent high dose NO treatment was safe, well tolerated and significantly reduced the bacterial and fungal load including several antibiotic resistant pathogens in the patients' airways. Additionally, this regimen reduced lung inflammation and significantly increased patients' lung function.

## Methods

### Study design and patients

Patients with CF attending the Comprehensive Cystic Fibrosis Center (CCFC) at the Robert-Bosch Krankenhaus (RBK), Klinik Schillerhöhe and the Children's Clinic of the University Hospital Tuebingen, Germany, were eligible to be included in the study if they were 18 years or older with values of forced expiratory volume in 1 s predicted (FEV<sub>1</sub>%) of <80 and >30 %, had evidence of chronic (≥6 months) bacterial lung infection at screening,

an oxygen saturation (SaO<sub>2</sub>) of >90 % and methemoglobin (MetHb) values of <3 %. Inhaled and oral antibiotic therapy were stopped in all patients 1 week before the first NO treatment. Patients were allowed to continue to receive mannitol (*n* = 1), pulmozyme (*n* = 2) or hypertonic saline inhalation (*n* = 5).

Exclusionary criteria included clinical deterioration (acute exacerbation), requirement with oxygen therapy, pregnancy, breast feeding and ineffective contraception, cardiac (right heart) or liver function insufficiency or hemoglobin values <13 g/dl.

Eight analyzable patients were enrolled to assess the safety of the NO treatment. All patients were analyzed in an intention to treat analysis (ITT) for safety. Efficacy analyses were performed in the ITT group and in the per protocol (PP) group, defined as patients who had 8 out of 10 treatment days and had a minimum of two out of three NO inhalations daily. A minimum of six patients was necessary for evaluation of the PP analyses.

Termination of treatment was required if at least one of the following criteria was met: (a) violation of the study protocol (missing study medication for 1 day); (b) intolerable and safety related side effects (arterial hypotension: systolic <90 mmHg; MetHb >5 %, SaO<sub>2</sub> <88 % and dyspnoea, lethargy); (c) acute pulmonary complications (pneumothorax, endotracheal or bronchial haemorrhage); (d) acute pulmonary exacerbations (two or more of the following criteria were considered to be an indication for a pulmonary exacerbation: increase in sputum volume, change in color or increased cough or increased malaise, fatigue, lethargy or anorexia, weight loss or decrease in pulmonary function by 10 % or more, or radiographic changes or increased dyspnoea); (e) any other condition, determined by the attending physician, that might jeopardize the patient's health if they continued to participate in the study. As a phase I safety study, neither patients nor investigators were blinded to the therapy. The patient identification list was kept exclusively by the investigating physician in the investigator site file. Monitoring for this study was provided by the Centre for Pediatric Clinical Studies at the Universitätsklinikum Tübingen.

The study was performed in accordance with the Declaration of Helsinki and under the regulations of Good Clinical Practice. The Ethics committee of the University of Tübingen and the Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte, BfArM) approved the clinical trial and written informed consent was obtained from all patients.

### Study procedures

Patients received 160 ppm of NO from an 800 ppm nitric oxide, balance nitrogen gas cylinder (Linde AG, Munich,

Germany) continuously mixed with room air and with maximum 1 liter per minute (lpm) of oxygen to deliver 160 ppm by inhalation via an experimental NO device designed for this study (NO-A, Maquet GmbH, Rastatt, Germany). NO was administered three times daily for two periods of five days each over two consecutive weeks. The duration of treatment for 10 days was chosen after discussion with the BfArM assuming that this time span might be adequate to treat multiresistant and long-living bacteria such as *M. abscessus*. Each treatment was for 30 min with a minimum time of 3 h between the treatments based on the NO study results in healthy individuals [22]. Patients were screened 7 days prior to treatment, treated with NO for 5 days as inpatients, followed by 2 days of treatment-free recreation (weekend) at home and treated as outpatients for another 5 days. Thereafter patients were followed up for 7 days. For patients' safety, heart frequency rate and O<sub>2</sub> saturation were continuously monitored during treatment, the concentration of NO and NO<sub>2</sub> was measured continuously in the gas stream and MetHb was continuously monitored transcutaneously (RAD57, Masimo, USA).

Study-related tests and examinations included the assessment of medical history data, vital parameters, laboratory parameters, information on therapy and data, relating to pathogen diagnostics. The following tests were performed in addition to the medically indicated tests and examinations. Blood gas analysis (pH, MetHb, pO<sub>2</sub>, pCO<sub>2</sub>) at day 1, prior, directly following NO inhalation and at 30 min following the end of NO inhalation at minimal 2 therapy cycles of 30 min. In the following days these analyses were carried out after all therapy cycles. In addition, the following hematologic analyses were carried out prior to the first treatment, after 5 days of therapy and at the end of the entire treatment period: leukocytes, hemoglobin, hematocrit, thrombocytes determinations, clinical chemistry (ALT, AST, urea, creatinine, CRP, IgG). If medically indicated, these analyses were carried out also at other visits. Furthermore, 5 ml blood was taken at days 1, 5 and 10 for the analysis of additional parameters in case of late adverse reactions which needed to be explored.

Following each initial daily NO administration, at least 1.5 ml of purulent sputum was collected from all CF patients for determination of microorganisms and white blood cell (WBC) counts on all treatment days. The vials containing the sputum were sent to the laboratory on a daily basis within 2 h of collection. Lung function (FVC, FEV<sub>1</sub>, MEF<sub>25-75</sub>) was tested spirometrically on all treatment days before and after treatment with an Asthma Monitor<sup>®</sup> AM1 device (Carefusion, Hoechberg, Germany). The increase of pulmonary inflammation was measured by assessing WBC and the neutrophil numbers in sputa. The effect of NO treatment on the microbial infection was measured by counting bacteria or fungi colony forming units (cfu) using

routine methods at baseline and at the end of treatment. A case report form was provided for patient documentation on a daily basis for all patients.

## Outcomes

The primary endpoint of the study was patient safety, which was determined by the course of methemoglobinemia in %, mean NO<sub>2</sub>, oxygen saturation, pH, PaO<sub>2</sub>, PaCO<sub>2</sub> and FEV<sub>1</sub>. Secondary endpoints were change of bacterial and fungal load after completion of the treatment from baseline, change in FVC, FEV<sub>1</sub> and MEF<sub>25-75</sub> from baseline and change of the serum (and sputum) parameters WBC, neutrophils, C reactive protein (CRP) and Immunoglobulin G (IgG).

## Statistical analysis

All parameters were evaluated as descriptive. The Student's *t* test or Wilcoxon was applied where appropriate. Unless otherwise stated, the variances were expressed as standard deviation. Because information about adverse effects of NO in CF patients in this first high dose study was not available from prior studies, we were unable to perform an a priori power analysis.

## Results

The prospective, monocentric, open labelled, clinical phase I study lasted for 7 months. Eight adult CF patients (Table 1) received gaseous NO by inhalation for 30 min, three times daily, at a concentration of 160 ppm for two periods of 5 days. Two patients who developed viral infections of the upper and lower respiratory tract, were excluded from the per-protocol (PP) analysis, while one patient developed a viral infection in the gastrointestinal tract, not related to the study drug.

## Inhaled NO Safety

No serious drug-related adverse events which might have caused termination of the study were observed. Furthermore, no patient had to stop NO treatment. A total of seven adverse events were recorded, four were deemed related to the study drug and diagnosed as transient xerostomia. Two patients developed a possibly drug related adverse event. Both patients developed a common cold of the upper and lower respiratory tract, followed by cough but were not febrile. Antibiotics were not given and identifying the viral pathogens were beyond the scope of this pilot trial. A third patient developed a viral infection in the gastrointestinal tract and assessed as un-related to the study drug.

**Table 1** Demographic data and baseline patient characteristics ( $n = 8$ )

Character	Amount
Patients—no.	8
Gender female—no. (%)	2 (25 %)
Age (years) <sup>a</sup>	34.6 ± 7.5
Weight (kg) <sup>a</sup>	59.1 ± 8.3
Blood pressure (mmHg), systolic–diastolic <sup>a</sup>	119 ± 9–74 ± 8
Heart rate (beats/min) <sup>a</sup>	77 ± 7.5
Pancreas insufficiency—no. (%)	7 (87.5)
Pulmonary colonization	
<i>P. aeruginosa</i> , non mucoid—no.	8
<i>P. aeruginosa</i> , mucoid—no.	1
<i>Stenotrophomonas maltophilia</i> —no.	1
<i>Staphylococcus aureus</i> —no.	2
Methicillin resistant <i>S. aureus</i> —no.	1
<i>Aspergillus fumigatus</i> —no.	1
<i>Aspergillus flavus</i> —no.	1
<i>Candida albicans</i> —no.	2
<i>Mycobacterium abscessus</i> —no.	2
<i>E. coli</i> /ESBL—no.	1
<i>Achromobacter dentrificans</i> —no.	1

<sup>a</sup> Values are mean ± SD

The infection started on day 8 of NO treatment after the patient had acquired a food-borne enteritis. NO treatment was postponed for 2 days but the patient completed all NO treatments successfully. MetHb increased but remained less than 3 % during the 30 min treatment period in 7/8 patients

and decreased thereafter to baseline levels after 3.5 h. In one female patient, MetHb reached the 3 % level after 25 min during each treatment resulting in cessation of the treatment after 25 min. Mean NO<sub>2</sub> was 4.0 ± 0.8 ppm during the 30 min NO treatment courses.

Oxygen saturation remained above 95 % in all patients. Arterial blood pressure did not decrease more than 5 % from mean systolic values and heart rates did not change significantly (Table 2). Furthermore, no changes were found in hemoglobin, thrombocytes, renal and liver laboratory parameters after NO treatment (Table 2).

### Anti-bacterial and fungal effect of NO therapy

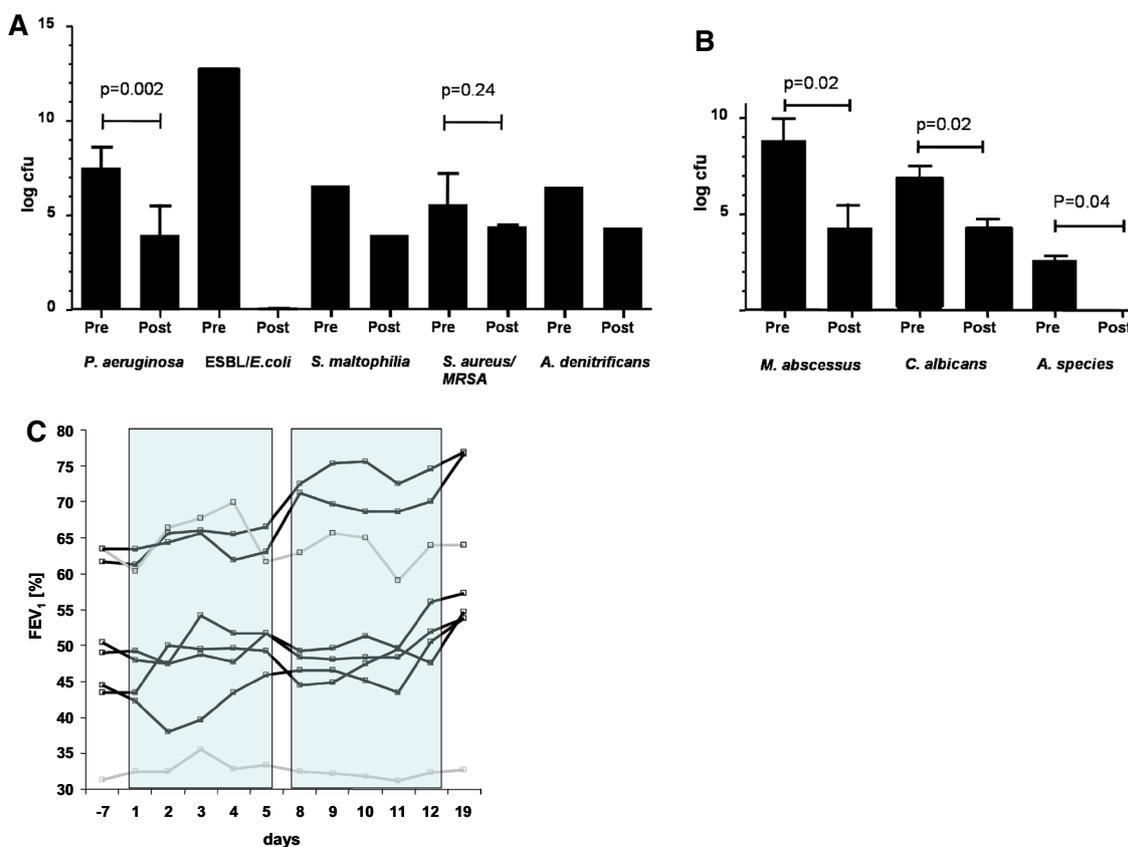
The ITT analysis revealed a significant mean reduction of the colony forming units (cfu) of all bacteria (log<sub>10</sub> cfu: pre: 7.4 ± 2.7; post: 3.8 ± 1.7;  $P = 0.0008$ ) and all fungi (log<sub>10</sub> cfu: pre: 5.6 ± 3.1; post: 2.6 ± 2.4;  $P = 0.0019$ ). The NO treatment reduced the cfu of various bacteria and fungi by several orders of magnitude (OM): *P. aeruginosa* by 3.5 OM, *E. coli* by 12 OM, *S. maltophilia* by 3 OM, *S. aureus*, including methicillin resistant *S. aureus* (MRSA) by 1 OM, *A. dentrificans* by 2 OM, *M. abscessus* by 4.5 OM, *C. albicans* by 2.5 OM, *A. fumigatus* and *A. flavus* by 2.5 OM (Fig. 1a, b). The extended broad spectrum β-lactamase (ESBL)-producing *E. coli* strain and both *Aspergillus* species were undetectable after therapy. The results show that 160 ppm NO treatment for two periods of 5 days, inhaled three times daily, had broad antimicrobial activity, including activity against antibiotic multi-resistant microorganisms.

**Table 2** Safety of NO in CF patients

Parameter	Before treatment	During treatment	After 10 days treatment	<i>P</i> value
Blood pressure, systolic—no. 25 (mmHg)	114.0 ± 12.2*	109.2 ± 14.4		0.09
Blood pressure, diastolic—no. 25 (mmHg)	70.5 ± 7.8	69.9 ± 8.3		0.38
Heart rate—no. 228 (beats/min)	89.2 ± 17.1	88.5 ± 15.6		0.31
NO <sub>2</sub> —no. 228 (ppm)	0	4.0 ± 0.8		<0.001
MetHb	0.6 ± 0.4	2.7 ± 0.4		<0.001
MMEF—no. 92 (%)	28.9 ± 16.2	28.5 ± 16.5		0.49
Leukocytes—no. 8 (1/μl)	10,269 ± 4768	10,269 ± 4105	11,095 ± 3486	0.70
Neutrophils—no. 8 (%)	72.5 ± 8.5	76.5 ± 6.8	72.5 ± 8.8	1.0
Platelets (1000/μl)	307 ± 51	328 ± 54	342 ± 57	0.21
Creatinine (mg/dl)	0.7 ± 0.1	0.8 ± 0.2	0.8 ± 0.2	0.46
AST (U/l)	24.9 ± 5.3	25.9 ± 8.6	25.1 ± 4.5	0.92
ALT (U/l)	25.5 ± 8.5	26.3 ± 10.3	26.4 ± 9.7	0.85
CRP (mg/dl)	1.2 ± 0.9	1.2 ± 1.3	1.5 ± 1.6	0.64
IgG (g/d)	13.3 ± 4.4	13.1 ± 5.9	14.3 ± 4.6	0.67

MetHb methemoglobin, MMEF maximal (mid-) expiratory flow, AST aspartate aminotransferase, ALT alanine aminotransferase, CRP C reactive protein, no. number of measured parameter

\* Values represent mean ± SD; the Student's *t* test was used for all statistical determinations



**Fig. 1** NO decreases the microbial pathogen load in the airways of cystic fibrosis (CF) patients and increases lung function. Microbial pathogens were quantified in sputum specimens of 8 CF patients before and after cumulative NO cycles by routine culture dilution techniques using agar plates, specific for the various pathogens. **a** Bacterial pathogens; **b** *Mycobacterium abscessus* and fungal pathogens. *P* values were calculated with the student's *t* test if more than

two microbial pathogens were present in sputum specimens of the 8 CF patients. **c** Forced expiratory volume 1 s % predicted (FEV<sub>1</sub>) was determined by spirometry in 8 CF patients receiving 160 ppm NO by inhalation for 2 × 5 days, trice daily. *Grey boxes* duration of NO treatment; *grey lines* patients acquiring viral lung infections during the treatment period

### Improvement in lung function after NO therapy

In the ITT analysis mean FEV<sub>1</sub> absolute and relative to baseline increased  $8.8 \pm 4.9$  and  $17.3 \pm 8.9$  % ( $P = 0.012$ ), respectively (Fig. 1c; Table 3). In the per-protocol (PP) analysis mean FEV<sub>1</sub> absolute and relative to baseline increased  $10.9 \pm 3.4$  and  $21.4 \pm 5.7$  % ( $P = 0.028$ ), respectively (Table 3). FVC absolute to baseline and relative to baseline increased in the ITT analysis to  $12.4 \pm 5.1$  and  $20.1 \pm 8.4$  % ( $P = 0.012$ ) and in the PP group to  $13.6 \pm 5.4$  and  $21.0 \pm 8.7$  % ( $P = 0.028$ ). MEF<sub>25-75</sub> absolute and relative to baseline increased in the ITT analysis to  $8.1 \pm 8.3$  and  $33.4 \pm 50.4$  % ( $P = 0.036$ ) and in the PP group to  $10.7 \pm 6.8$  and  $49.2 \pm 46$  % ( $P = 0.046$ ) (Table 3). These data demonstrate the potential of NO to increase lung function to a degree rarely observed after antibiotic therapy courses in CF patients.

NO treatment reduced pulmonary inflammation as demonstrated by a significant reduction in sputum leukocyte

numbers (Table 3). No differences were found before and after NO treatment in several inflammatory serum parameters such as leukocytes, neutrophils, CRP and IgG (Table 3).

In summary, our data reveal NO as a novel treatment option for CF lung disease which reduces the microbial load and lung inflammation thereby increasing lung function.

### Discussion

This is the first in human study to investigate the feasibility and safety of intermittent 160 ppm NO in CF patients. We observed that the novel treatment regimen is safe and led to a clinically relevant mean reduction of bacterial and fungal numbers, a clinically relevant reduction in lung inflammation and a clinically relevant increase in lung function. These observations of safety and tolerance to multiple daily

**Table 3** Secondary parameters at baseline and post treatment in the intention-to-treat (ITT) and the per protocol (PP) analysis

Parameter	ITT (n = 8)			PP (n = 6)		
	Pre treatment	Post treatment	P value	Pre treatment	Post treatment	P value
FVC (%)	63.3 ± 13*	75.6 ± 14.6	0.012	64.9 ± 8.4	78.5 ± 10.8	0.028
FEV <sub>1</sub> (%)	49.9 ± 11.2	58.7 ± 14.3	0.012	51.2 ± 9.0	62.2 ± 11.4	0.028
MEF <sub>25–75</sub> (%)	28.6 ± 15.6	36.7 ± 18.6	0.036	26.1 ± 11.5	36.7 ± 13.4	0.046
WBC in serum (× 10 <sup>3</sup> /μl)	10.81 ± 4.2	11.1 ± 3.5	0.36	10.86 ± 4.4	11.22 ± 3.4	0.37
Neutrophils in serum (%)	73.0 ± 8.2	72.5 ± 8.2	0.41	73.8 ± 8.8	74.2 ± 8.8	0.45
CRP (mg/dl)	2.72 ± 3.8	1.54 ± 1.6	0.24	3.15 ± 4.4	1.12 ± 1.1	0.17
IgG (g/l)	13.3 ± 4.4	14.3 ± 4.6	0.12	12.6 ± 3.8	12.8 ± 4.1	0.34
WBC in sputa (× 10 <sup>3</sup> /μl)	62.2 ± 47.7	20.9 ± 13.5	0.01	62.2 ± 45.4	19.5 ± 10.2	0.03
Neutrophils in sputa (%)	40.8 ± 17.4	26.6 ± 16.1	0.0001	43.7 ± 13.8	28.2 ± 16.2	0.0002

FVC forced vital capacity, FEV<sub>1</sub> forced expiratory volume in 1 s predicted, MEF maximal expiratory flow, WBC white blood cells, CRP C-reactive protein, IgG immunoglobulin G, P was determined using Wilcoxon test

\* Values are mean ± SD

inhalations of 160 ppm NO for 30 min are consistent with those reported by Miller et al. [23] in a cohort of healthy adults.

Previous in vitro studies, utilizing a variety of NO donors or directly gaseous NO, suggested that NO possesses antimicrobial activity against a wide variety bacteria, viruses, helminthes and parasites [18, 23], including mucoid *P. aeruginosa* present in biofilms in CF airways [24]. Mechanisms of action comprise interference with DNA repair mechanisms, binding to heme or thiol containing metabolic proteins or damage of membrane lipids [25, 26]. Furthermore, NO modulates the host immune response by activating neutrophils, macrophages or epithelial cells [19]. The findings that aerosolized administration of 160 ppm NO was highly bactericidal in a rat model of acute *P. aeruginosa* pneumonia [22], and neither toxic nor mutagenic for human cell lines [27], suggested that this treatment regimen may represent a promising therapeutic approach for patients with bacterial lung infections.

NO is an approved drug for term infants at concentration of ~20 ppm, and can be inhaled continuously for weeks [28]. However, at that concentration, antimicrobial effects of NO have not been reported previously. Methemoglobinemia is the most identified significant side effect related to inhaled NO therapy. High MetHb% may result in hypoxemia in patients with already compromised oxygenation. Continuous inhalation of even 60–80 ppm NO results in the increase of MetHb% levels to as high as 12 % [28]. The half-life of MetHb is estimated to be 60 min in humans, being reduced by MetHb reductase into nitrites/nitrates and ultimately excreted in the urine. MetHb formation is a single order pharmacokinetic function and it has been demonstrated that the rise in MetHb during breathing of 160 ppm NO in 30 min is approximately 1 %. This formation and half-life clearance report has been validated in prior safety

studies so that that 160 ppm NO could be delivered for 30 continuous minutes every 4 h without increasing MetHb % to rise above 3 % and that it returns to baseline prior to the next 30 min of NO therapy [23]. Results from this study support this theory, in that MetHb remained below 3 % in all but one female patient. In her case, the MetHb threshold level of 3 % was reached after 25 min of NO inhalation. It is unknown why this occurred. Whether there is a gender effect remains an open question.

Because inhalation of 160 ppm NO for longer periods of time would unequivocally lead to formation of toxic NO<sub>2</sub> levels [29], MetHb and unacceptable hypoxemia, intermittent NO inhalation provides an alternative. This NO regimen, which allows 3.5 h between 30 min treatments courses to let MetHb and NO<sub>2</sub> reach baseline values, has been shown to be safe in a study of 10 healthy individuals even after multiple NO treatment courses [23].

In this study, the most common side effect, which was reported four times, was xerostomia. This was also reported in a previous NO study in healthy adults in one subject receiving NO therapy and is most likely due to administration of with a mouth piece for 30 min [23]. NO<sub>2</sub> and NO are free radicals which have been reported to cause irritation and lung damage [28]. Inflammation can be indirectly measured in the blood and sputum by assaying inflammatory cytokine and chemokine levels such as myeloperoxidase, TNF-alpha, IL-6 and IL-8, but was not done in this pilot study and must be done in further trials. Clinically, acute inflammation can be measured by coughing episodes but more quantitatively with pulmonary function testing. In both this study and the healthy population study, clinical signs and objective measurements of inflammation were monitored but not observed during and post NO inhalation. It is possible that acute inflammation may have been masked by the acute bronchodilatory effects of NO. Should

future studies require NO delivery exceeding 30 days, long term lung function monitoring should be considered.

Although not powered for efficacy endpoints, our study convincingly showed that 160 ppm NO inhaled by CF patients significantly reduces the cfu of various bacteria and fungi by several orders of magnitude. This is particularly noteworthy with regard to pathogens displaying intrinsic or acquired antibiotic resistance as present in the ESBL-producing *E. coli* strain, the *M. abscessus* strain and the *Aspergillus* species. The large reductions of bacterial numbers led consequently to reduced pulmonary inflammation and to increases in the lung function parameter FEV<sub>1</sub> from baseline to a degree seldom observed after antibiotic therapy courses in CF patients. Based on the small patient numbers in this study, this increase in the lung function has to be confirmed in further studies. Antibiotics exert their effect on organism specific targets, whereas NO appears to have a multiplicity of targets that are non-organism specific. NO's non-specificity is most likely attributed to its oxidative and nitrosylating effects. NO eradicates microbes by nitrosylating their heme- or thiol-containing essential metabolic proteins [30, 31]. To date bacteria, viruses and fungi have all been susceptible to NO [18–21]. This may be due to NO-nitrosylation which interferes with RNA replication and DNA repair mechanisms; damages cellular structure and function; and modulates the host immune response [18–22].

Taken together, we hypothesize that multiple, short duration high-dose inhaled administrations of NO, if proven safe and effective, may constitute a novel strategy to improve the therapy for chronic bacterial and fungal lung infections in CF patients. Other patient groups at risk for microbial lung infections may also benefit from this novel treatment such as those with mycobacterial infections, chronic obstructive pulmonary disease and ventilator associated pneumonia. NO therapy may avoid adverse effects caused by repeated courses of antibiotic treatment, may improve quality of life, as well as the prognosis and life expectancies for infected patients. Based on the results of this pilot study we believe that further studies are warranted and are currently in progress.

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#### Compliance with ethical standards

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