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Short Communication

Lower exhaled nitric oxide in infants with Cystic Fibrosis compared to healthy controls

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Abstract

Exhaled nitric oxide (FE_{NO}) is a well-known, non-invasive airway biomarker. In patients with Cystic Fibrosis (CF) FE_{NO} is decreased. To understand if reduced FE_{NO} is primary related to Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) dysfunction or an epiphenomenon of chronic inflammation, we measured FE_{NO} in 34 infants with CF prior to clinical symptoms and in 68 healthy controls. FE_{NO} was lower in CF compared to controls ($p = 0.0006$) and the effect was more pronounced in CF infants without residual CFTR function ($p < 0.0001$). This suggests that FE_{NO} is reduced in CF early in life, possibly associated with underlying CFTR dysfunction.

1. Introduction

The fractional concentration of exhaled nitric oxide (FE_{NO}) is a well-known biomarker for airway inflammation and elevated in a number of inflammatory disorders of the lung [1]. In patients with Cystic Fibrosis (CF) and predominantly neutrophilic airway inflammation, FE_{NO} is decreased [2–4]. The following underlying causes have been discussed: (i) reduced NO synthase isoenzyme (NOS) expression, (ii) lack of NOS substrates, (iii) reduced NOS function through endogenous inhibitors (e.g. methylated arginine derivatives and polyamines), (iv) NO decomposition by bacterial reductases or neutrophilic myeloperoxidase, or (v) impaired NO diffusion through viscous mucus [1,5–9]. Reduced levels of NO or NOS have been related to a number of adverse effects, such as increased airway narrowing, reduced ciliary motility and susceptibility to infections [2,10].

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Table 1
Study population and FE_{NO} measurements.

	Healthy infants (n = 68)	Infants with CF (n = 34)
Gender, female	33 (49%)	12 (35%)
Age at study date, wk	5 (+/−1.2)	8 (+/−2.5)
Gestational age, wk	39.5 (+/−1.4)	38.7(+/−1.7)
Length at birth, cm	50 (+/−2.3)	50 (2.8)
Weight at birth, g	3400 (+/−567)	3310 (+/−569)
Weight at study date, g	4430 (+/−485)	4970 (+/−1413)
CF mutation[a]:		
No residual CFTR function (class I and/or II)		19 (56%)
Residual CFTR function (class I/II and III-VI)		7 (21%)
Unknown CFTR function		8 (23%)
FE_{NO} ppb	17.0 (+/−5.0)	13.7 (+/−5.3)
FE_{NO} ppb median (IQR)	16.4 (13.8–18.7)	12.2 (10.2–16.3)
V′NO nl/min	46.3 (+/−11.5)	38.5 (+/−15.7)
Flow exp. ml/s	47.8 (+/−11.0)	48.2 (+/−11.1)
Respiratory Rate (1/min)	43.1 (+/−8.5)	43.0 (+/−8.5)

Results are displayed in number (%) and mean (+/− SD) if not stated otherwise. wk = weeks.

[a] CF infants were grouped in 1: two known copies of class I and/or II mutations 2: at least one copy of a class III-VI mutation 3. > = 1 mutation not classified or unknown mutation. All children in group #3 however had two copies of disease causing mutations.

Absent or residual function of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein in CF patients results in insufficient NOS induction, which however appears reversible [11]. Reduced FE_{NO} normalizes in patients with CF after treatment with Ivacaftor, one of the first approved CFTR-targeting drugs for certain gating mutations [12]. This suggests that decreased FE_{NO} in CF is not an epiphenomenon of chronic inflammation or infection, but might be reduced in CF airways early in life, possibly associated with the defect in CFTR.

In order to understand whether reduced FE_{NO} in CF airways can be detected early in life, FE_{NO} measurements in a very young age and before the onset of first apparent infections need to be explored. We thus measured FE_{NO} in infants with CF and healthy controls at five to twelve weeks of age.

2. Methods

We enrolled infants with CF diagnosed by new-born screening and contemporary healthy infants aged five to twelve weeks, matched 1:2 based on season of birth and sex, from two ongoing birth cohort studies, the Swiss Cystic Fibrosis Infant Lung Development (SCILD) cohort [13] and the Basel Bern Infant Lung development (BILD) cohort [14] born between 2011 and 2015. Exclusion criterion was a history of respiratory symptoms suggesting upper- or lower respiratory tract infection prior to the study. Clinical examination was normal in all infants. Infants with asymptomatic bacterial colonization were not excluded (n = 5). All lung function measurements were performed in the study centres Basel (n = 1) and Bern, and infants were between five and twelve weeks of age. FE_{NO} measurements were performed between 9:30 a.m. and 3.30 p.m. during tidal breathing, in regular, quiet sleep as previously described [15–18]. In brief, FE_{NO} measurements were obtained online using a tight fitting face mask (covering nostrils and mouth) with a rapid response chemiluminescence analyser, a previously validated device (CLD 77; Eco Medics AG, Duernten, Switzerland; analysis software: WBreath 3.28, ndd, Zurich, Switzerland). To avoid contamination by ambient NO, we used NO-free air for inspiration. FE_{NO} was sampled breath-by-breath during the third quartile of expiration and averaged across 100 consecutive breaths. This was validated to be the measurement set up with the least variability of FE_{NO} [18]. As FE_{NO} is flow dependent, correcting for expiratory flow was performed as described previously [17,18]. Results are thus presented for both FE_{NO} and V′NO (NO output = FE_{NO} multiplied with corresponding expiratory flow), which were the primary outcomes. Additionally, flow and respiratory rate were secondary outcomes. We compared NO levels between healthy controls and infants with CF. Subsequently, patients were stratified into CFTR groups with (i) no residual function (two copies of class I and/or class II mutations), (ii) residual function (infants with at least one copy of a class III-VI mutation, and (iii) unclassified mutations. As FE_{NO} data were not perfectly normally distributed, we illustrate average estimates in both mean and median. We performed both Wilcoxon-Man-Whitney test and linear regression after log transformation of variables and adjusted for possible confounders

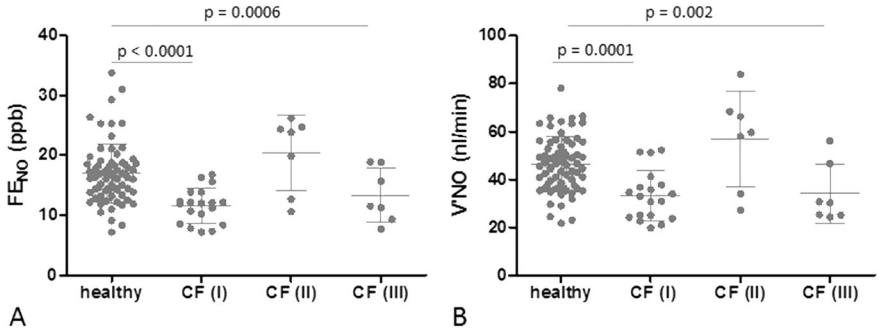

Fig. 1. (A) FE_{NO} and (B) V′NO measurements in healthy infants (n = 68) and infant with CF with (I) no CFTR residual function (n = 19), (II) with CFTR residual function (n = 7) and (III) unknown CFTR function (n = 8). Each dot symbolizes one infant. Lines indicate mean +/−SD.

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