Airway and alveolar nitric oxide production, lung function, and pulmonary blood flow in sickle cell disease

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BACKGROUND: Children with sickle cell disease (SCD) often have obstructive lung function abnormalities which could be due to asthma or increased pulmonary blood volume; it is important to determine the underlying mechanism to direct appropriate treatment. In asthmatics, exhaled nitric oxide (F_{eNO}) is elevated. F_{eNO}, however, can also be raised due to increased alveolar production. Our aim, therefore, was to determine if airway or alveolar NO production differed between SCD children and ethnic and age-matched controls.

METHODS: Lung function, airway NO flux and alveolar NO production, and effective pulmonary blood flow were assessed in 18 SCD children and 18 ethnic and age-matched controls.

RESULTS: The SCD children compared to the controls had a higher respiratory system resistance ($P = 0.0008$), alveolar NO production ($P = 0.0224$), and pulmonary blood flow ($P < 0.0001$), but not airway NO flux. There was no significant correlation between F_{eNO} and respiratory system resistance in either group, but in the SCD children, there were correlations between alveolar NO production ($P = 0.0006$) and concentration ($P < 0.0001$) and pulmonary blood flow.

CONCLUSION: Airway NO flux was not elevated in the SCD children nor correlated with airways obstruction, suggesting that airways obstruction, at least in some SCD children, is not due to asthma.

It is estimated that approximately 250,000 children are born with sickle cell disease (SCD) each year (1). The majority of children with SCD in developed countries can expect to survive to adulthood (2) but may then suffer severe morbidity including chronic hypoxia and pulmonary hypertension (3). Whereas restrictive lung function abnormalities are common in adults, obstructive lung function abnormalities are more frequently reported in children (4), but the etiology of the latter remains unclear. It is possible that the obstructive lung function abnormalities may be due to asthma. An increased prevalence of asthma was reported in one study of SCD children (5), but other studies have indicated a similar incidence to that of non-SCD populations (6,7). Another

explanation for the airways obstruction in SCD is the hyperdynamic pulmonary circulation due to a raised cardiac output resulting from chronic anemia (8). In children with SCD, we have demonstrated that pulmonary capillary blood volume is increased compared to matched controls and correlated with airflow obstruction (9). In a study of vascular abnormalities on high-resolution computed tomography scanning, we used two quantitative measures to assess vessel dilatation (the segmental artery-bronchus (A/B) ratio and the total cross-sectional area of all pulmonary vessels less than 5 mm in diameter (cross-sectional area < 5 mm%)) (10). Increases in segmental A/B ratio and cross-sectional area < 5 mm% were independently linked to reductions in forced expiratory volume in 1 s (FEV_1), vital capacity (VC), and forced expiratory flow between 25 and 75% of VC exhaled (FEF_{25-75}) and to increased respiratory system resistance and residual volume:total lung capacity (RV:TLC). Furthermore, small vessel dimensions correlated with reduced hemoglobin concentration and increased cardiac output (10). Those data suggest that the obstructive lung function abnormalities were related to a hyperdynamic circulation.

Measurement of exhaled nitric oxide (F_{eNO}), which is elevated in asthma due to enhanced expression of inducible nitric oxide synthase in inflamed airways, might help to further elucidate whether asthma is increased in SCD children. In one study, F_{eNO} was reduced in SCD patients (11), a possible explanation is reduced nitric oxide (NO) bioavailability resulting from chronic hemolysis and NO scavenging by the cell free hemoglobin (12). A reduced F_{eNO}, however, has not been found in other cohorts (13,14). F_{eNO} has also been shown to be elevated in patients with atrial septal defects or liver cirrhosis. Both conditions are associated with a hyperdynamic pulmonary circulation and F_{eNO} correlated with increased pulmonary blood flow (15,16). In patients with liver cirrhosis, the enhanced NO output was shown to be of alveolar and not airway origin (17). Whether the hyperdynamic circulation in SCD patients (8) influences their F_{eNO} levels has not been investigated.

In individuals with SCD, F_{eNO} has been previously measured at a single fixed exhalation flow of 50 ml/s (11,13) but measurements at multiple exhalation flows allow partitioning into

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flow-independent airway and alveolar components (18,19). Using such a technique, SCD children were found to have an elevated airway nitric NO flux, but the alveolar NO concentration did not differ significantly from that found in healthy controls (14). A limitation of that study (14), however, was that the children in the control group were not matched for race and ethnic differences have been reported for F_{eNO} indices (20–22). Furthermore, as the transfer factor for NO (DLNO) was not measured (14), it was not possible from those results to determine whether any elevation might be due to passive accumulation due to gas exchange impairment or reflected increase in alveolar production. Thus, it is important that flow-independent F_{eNO} parameters obtained using the multiple exhalation flow technique are measured in SCD patients and compared with appropriately matched controls to reliably determine whether or not F_{eNO} is elevated in SCD children and whether any elevation of F_{eNO} is due to increased airway NO flux or to increased alveolar NO production due to a hyperdynamic pulmonary circulation.

We, therefore, aimed to determine if airway NO flux and alveolar NO production differed between children with SCD and ethnic-matched controls and whether airway NO flux correlated with airways obstruction and alveolar NO production to a hyperdynamic pulmonary circulation. Such data would inform the debate as to whether airways obstruction in SCD is due to asthma and hence treatment strategies.

RESULTS

Subjects

Eighteen children with SCD and 18 controls were assessed. The SCD children and the controls were of similar age and sex (**Table 1**). Two patients with SCD and one control had a physician diagnosis of asthma. One of the SCD patients with asthma was receiving inhaled corticosteroids. Nine SCD children had a history of acute chest syndrome and five were on hydroxyurea therapy; none were undergoing regular blood transfusions.

Lung Function, F_{eNO}, and Effective Pulmonary Blood Flow

The SCD children compared to the controls had a significantly lower FEV$_1$ ($P < 0.0001$), VC ($P < 0.0001$), FEF$_{25-75}$ ($P = 0.0071$), FEV$_1$:VC ($P = 0.0315$), TLC ($P = 0.0024$), and SpO$_2$ ($P = 0.0023$) and a significantly higher R$_5$ ($P = 0.0008$) and K$_{CO}$ ($P = 0.0114$) (**Table 1**). In the SCD group, three patients (17%) had restrictive, two obstructive (11%), and two mixed (11%) ventilatory defects. Three SCD patients (17%) had an isolated reduction in DLCO. None of the controls had a restrictive defect and one had an obstructive pattern. One control had an isolated reduction in DLCO.

There were no significant differences between the groups with regard to $F_{eNO, 50 ml/s}$ or total maximal airway NO flux ($J'_{aw,NO}$) (**Table 2**). Compared to the control group, the SCD patients had a significantly higher alveolar NO concentration ($C_{A,NO}$) ($P = 0.0007$), rate of alveolar NO production ($V'_{A,NO}$) ($P = 0.0224$), effective pulmonary blood flow (Q_{pulm}) ($P = 0.0006$), and pulmonary blood flow index (QI_{pulm}) ($P < 0.0001$).

Table 1. Demographics and lung function by sickle cell disease status

N	Sickle cell disease children	Controls	
	18	n = 18	P value
Sex (female n (%))	9 (50%)	9 (50%)	1.0000
Age (years)	16.0 (9–18)	17.0 (11–18)	0.2310
[Hb] (g/dl)	8.3 (6.0–10.3)	-	-
FEV$_1$	79.1(47.5–105.8)	104.5(78.6–124.5)	<0.0001
VC	85.1 (48.6–107.8)	107.5(80.2–125.6)	<0.0001
FEF$_{25-75}$	58.5 (29.2–138.2)	92.4(53.5–153.9)	0.0071
FEV$_1$:VC	91.0 (75.2–104.5)	98.8(87.3–112.1)	0.0315
TLC	83.1 (73.8–106.5)	102.5(85.5–125.3)	0.0024
RV	85.1 (31.8–125.3)	93.0(58.11–155.3)	0.1461
D$_L$CO	88.5 (65.0–122.0)	87.1(66.1–127.7)	0.5841
K$_{CO}$	97.4 (70.0–132.0)	86.1(69.0–115.1)	0.0114
R$_5$	145.6 (107.8–234.1)	111.7(67.2–168.6)	0.0008
SpO2* (%)	95 (89–99)	98(96–99)	0.0023
D$_L$NO* (in ml/min/mHg)	62.7 (37.9–83.8)	85.3(44.3–113.3)	0.0002

Results are presented as median (range) and the lung function data expressed as a percentage of the predicted value unless otherwise indicated*. –indicate that data were not measured.
FEV$_1$, forced expiratory volume in 1 s; RV, residual volume; TLC, total lung capacity; VC, vital capacity.

Table 2. F_{eNO} and pulmonary blood flow measurements

N	Sickle cell disease children	Controls	
	18	18	P value
$F_{eNO,50 ml/s}$	14.7 (1.6–92.3)	14.6 (1.2–56.1)	0.7569
$J'_{aw,NO}$	33.5 (6.1–403.6)	60.8 (1.4–319.1)	0.3038
$C_{A,NO}$	7.0 (1.3–7.3)	2.5 (0.6–5.9)	0.0007
$V'_{A,NO}$	319.0 (57.3–663.7)	151.0 (31.8–447.1)	0.0224
Q_{pulm}	7.80 (4.25–10.85)	5.50 (3.55–7.70)	0.0006
QI_{pulm}	4.97 (3.77–6.82)	3.29 (2.48–4.50)	<0.0001

In the SCD children and the controls, no significant correlations were observed between F_{eNO} indices and R$_5$ or FEV$_1$:VC (**Table 3**). In the SCD group, Q_{pulm} was positively correlated with $F_{eNO,50 ml/s}$ ($P = 0.036$), $C_{A,NO}$ ($P = 0.0343$), and $V_{A,NO}$ ($P = 0.0002$) (**Table 4**). QI_{pulm} was positively correlated with F_{eNO} ($r = 0.591$, $P = 0.0112$), $C_{A,NO}$ ($r = 0.742$, $P = 0.0006$), and $V_{A,NO}$ ($r = 0.806$, $P < 0.0001$) (**Figure 1**). No significant correlations were seen between F_{eNO} indices and Q_{pulm} or QI_{pulm} in the control group (**Table 4**). In the SCD children, hemoglobin concentration was correlated with QIpulm ($r = -0.56$, $P = 0.0157$) and alveolar NO concentration ($r = -0.46$, $P = 0.0488$).

DISCUSSION

We have demonstrated that children with SCD compared to controls had an elevated alveolar nitric oxide concentration and increased alveolar production, but their maximal airway

Table 3. Correlations between F_{eNO} and airflow obstruction assessment

Sickle cell disease children			
		R	*P*
$F_{eNO,50l/s}$	FEV_1:VC	−0.340	0.1681
$F_{eNO,50l/s}$	R5	0.286	0.2493
$J'_{aw,NO}$	FEV_1:VC	−0.161	0.5139
$J'_{aw,NO}$	R5	0.420	0.0840
$C_{A,NO}$	FEV_1:VC	−0.179	0.4769
$C_{A,NO}$	R5	−0.214	0.3931
Controls			
$F_{eNO,50l/s}$	FEV_1:VC	−0.311	0.2234
$F_{eNO,50l/s}$	R5	−0.245	0.3417
$J'_{aw,NO}$	FEV_1:VC	−0.220	0.3805
$J'_{aw,NO}$	R5	−0.251	0.3153
$C_{A,NO}$	FEV_1:VC	−0.094	0.7108
$C_{A,NO}$	R5	0.267	0.2834

FEV_1, forced expiratory volume in 1 s; VC, vital capacity.

Table 4. Correlations between F_{eNO} and pulmonary blood flow measurements

Sickle cell disease children			
		r_s	*P*
Q_{pulm}	$Fe_{NO,50l/s}$	0.496	0.036
QI_{pulm}	$Fe_{NO,50l/s}$	0.591	0.0112
Q_{pulm}	$J'_{aw,NO}$	0.328	0.1834
QI_{pulm}	$J'_{aw,NO}$	0.290	0.2423
Q_{pulm}	$C_{A,NO}$	0.500	0.0343
QI_{pulm}	$C_{A,NO}$	0.742	0.0006
Q_{pulm}	$V'_{A,NO}$	0.761	0.0002
QI_{pulm}	$V'_{A,NO}$	0.806	<0.0001
Controls			
Q_{pulm}	$Fe_{NO,50l/s}$	−0.1030	0.7042
QI_{pulm}	$Fe_{NO,50l/s}$	−0.1235	0.6485
Q_{pulm}	$J'_{aw,NO}$	−0.1511	0.5631
QI_{pulm}	$J'_{aw,NO}$	−0.1680	0.5192
Q_{pulm}	$C_{A,NO}$	0.1804	0.4885
QI_{pulm}	$C_{A,NO}$	0.0233	0.9293
Q_{pulm}	$V'_{A,NO}$	0.4256	0.0886
QI_{pulm}	$V'_{A,NO}$	0.1936	0.4550

Figure 1. Alveolar nitric oxide production and the pulmonary blood flow index in sickle cell disease children (■). The line represents the least-square linear regression best fit; the shaded areas are the 95% confidence interval for the fit.

and SCD patients were of African or Caribbean ethnic origin, whereas Radhakrishnan recruited controls of various ethnicities, the majority of whom were Caucasian. In adults with SCD, Girgis *et al.* (11) reported a reduction in F_{eNO} 50 ml/s but used a predominantly Caucasian control group (comprising 9 African-American, 1 Asian, and 20 Caucasian subjects) (11).

Our results contrast with those from patients with cystic fibrosis and primary ciliary dyskinesia in whom airway NO is reduced compared to controls and the reduction in exhaled NO linked to more severe airway obstruction (23,24). In this study, we found no significant correlations between F_{eNO} indices and R5 or FEV_1:VC. A larger study (13) reported, as we now do, no difference in $F_{eNO50\,ml/s}$ when 50 SCD children were compared to 50 ethnic-matched controls. Furthermore, airflow obstruction, as evidenced by a reduced FEV:VC was not associated with increased methacholine sensitivity or elevated F_{eNO} (13). Those results (13) led the authors to hypothesize that sickling within the bronchial circulation may lead to airway ischemia and mucosal injury with the subsequent development of fibrosis and progressive airway obstruction. Recent studies have suggested that airflow obstruction seen in SCD at least in certain patients may reflect increased pulmonary capillary blood volume rather than asthma (9,10).

To the best of our knowledge, this study is the first to assess the relationship between pulmonary hemodynamics, F_{eNO}, maximal airway NO flux, and alveolar NO concentration/production in children with SCD. Our findings that alveolar NO production and concentration were correlated with effective pulmonary blood flow and alveolar NO production was elevated in the SCD group, despite a reduced D_LNO suggest that the elevated alveolar NO production was associated with a hyperdynamic circulation in SCD patients. In patients with

nitric oxide flux did not differ from that of ethnic-matched controls. Alveolar production and NO concentration were correlated with effective pulmonary blood flow in the SCD group but not in the controls, suggesting a link between alveolar NO production and a hyperdynamic circulation. Our results differ from those of Radhakrishnan *et al.* (14) who found that airway NO flux and exhaled NO at a flow of 50 ml/s but not alveolar NO production was elevated in SCD patients. Those differences may be due to a number of factors. All our controls
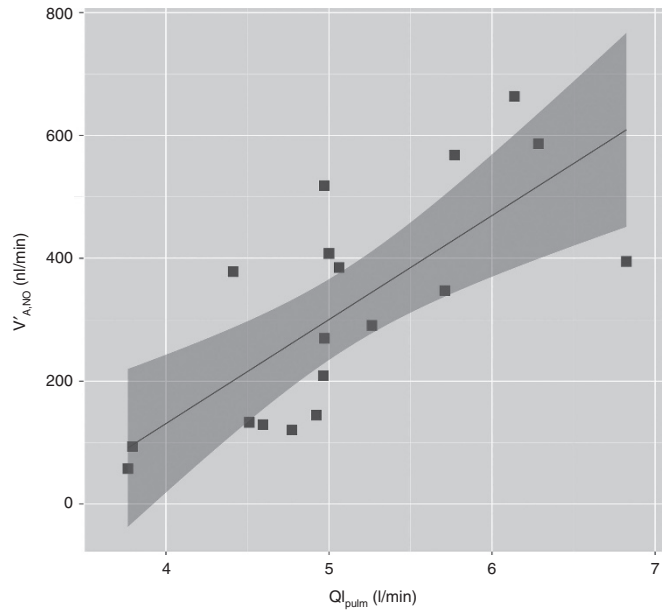
liver cirrhosis and hepatopulmonary syndrome, alveolar NO concentration and the rate of alveolar NO production were elevated compared to healthy controls (15) and positively correlated with cardiac index (cardiac output normalized for body surface area) measured during right-heart catheterization. F_{eNO} was also elevated in patients with an atrial septal defect (16); in addition, both pulmonary blood flow, exhaled NO concentration and plasma nitrate levels fell significantly in children and adults after surgical repair of the atrial septal defect. Although a different method for NO measurement was used, the F_{eNO} concentrations before atrial septal defect closure were similar to the alveolar NO production levels observed in this study. We have demonstrated a significant correlation between effective pulmonary blood flow and the pulmonary blood flow index and alveolar NO production. We, therefore, suggest that previously reported elevated F_{eNO} levels in SCD children (14) may be due to an increase in alveolar nitric oxide production.

This study has strengths and some limitations. Strength of this study was the use of multiple-flow exhaled NO measurements, which enabled the maximal airway NO flux and alveolar NO concentration to be assessed. We also measured D_LNO and were therefore able to demonstrate increased alveolar NO production. We used ethnic-specific reference values for spirometric results. Although static lung volume and impulse oscillometry results were related to reference ranges derived from Caucasian subjects, the two groups were matched for ethnic origin and the same reference ranges were used in both groups, thus the comparisons between them were valid. All of the SCD children and the controls were African or Caribbean, thereby avoiding confounding from ethnic differences in exhaled NO (20–22). It should also be noted that the sample size was relatively small in this study, which may have limited our ability to detect subtle differences between the SCD and control groups. Nevertheless, highly significant differences were observed for exhaled NO and lung function results. We used a noninvasive method to measure pulmonary blood flow rather than pulmonary artery catheterization or measurements derived from CT pulmonary angiography. The method and device we used has been previously validated in children (25) and does not involve exposure to ionizing radiation, a consideration particularly pertinent when testing control children.

Airway NO flux did not differ between the SCD children and ethnic-matched controls and was not related to the airflow obstruction demonstrated in the SCD children. Those results suggest that airway obstruction at least in certain SCD children is not due to asthma.

METHODS

Study Design

African-Caribbean children with SCD (homozygous for sickle cell hemoglobin) were recruited. Ethnic matched children, who were either siblings of the SCD children or from local schools, were recruited as controls. The controls were age matched to the SCD years such that their age was within 2 y of the SCD children. Only children over 7 y of age were recruited as they were likely to be able to complete all the lung function tests.

The study was conducted in the Amanda Smith Unit at King's College Hospital. No child underwent testing within 2 wk of an upper respiratory tract infection or an SCD child within a month of suffering a vaso-occlusive crisis. A history was taken of past and current respiratory symptoms and medication for respiratory problems. Standing height was measured using a wall-mounted stadiometer (Holtain, Crymych, Dyfed, UK), and weight using electronic weighing scales (Seca, Birmingham, UK). This study was conducted in accordance with the amended Declaration of Helsinki. The study was approved by the King's College Hospital Research Ethics Committee (approval number (08/H0808/29) and parents gave informed written consent for their child to take part.

Lung Function Measurements

Subjects were assessed while wearing a nose-clip and breathing through a mouthpiece. Respiratory system resistance at a frequency of 5 Hz (R_5) was measured during a 90 s period of tidal breathing using impulse oscillometry (IOS, Jaeger Masterscreen IOS, Carefusion, Basingstoke UK). The results were expressed as the percent predicted for height (26). The mean of two measurements within 10% of each other was reported. IOS was performed first to avoid changes in bronchial smooth muscle tone caused by deep inspiration. Exhaled NO was measured before the remaining lung function tests were performed as recent forced expiration can influence F_{eNO}.

Spirometry, static lung volumes using whole-body plethysmography and gas transfer for carbon monoxide were assessed according to American Thoracic Society/European Thoracic Society criteria (27–29). Forced expiratory volume in 1 s (FEV_1), VC, mean maximum expiratory flow (FEF_{25-27}), TLC, residual volume, transfer factor for carbon monoxide (DLCO), and transfer coefficient (K_{CO}) were assessed and the results expressed as the percent predicted for height (30,31). Ethnic-specific reference equations were not available for static lung volumes or gas transfer; therefore the predicted values were adjusted using appropriate correction factors (32). The transfer factor for nitric oxide (DLNO) was obtained by the addition of 40 ppm NO to the inspired gas mixture prior to commencing the single-breath DLCO measurement (33). Measurements were performed using a pneumotachograph-based system (Jaeger Masterscreen PFT, Carefusion, Basingstoke, UK). Patients were diagnosed with a restrictive abnormality if their TLC was less than the lower limit of normal with a normal FEV_1:VC, an obstructive abnormality if their FEV_1:VC was less than lower limit of normal and a mixed pattern if both TLC and FEV_1:VC were less than the lower limit of normal (34).

F_{eNO} Measurements

Exhaled nitric oxide was assessed according to American Thoracic Society/European Thoracic Society criteria (35). Exhalation was performed at target flows of 50, 100, 150, and 350 ml/s against a fixed resistance to maintain velo-pharyngeal closure. Expiratory flow was recorded using an integral flow meter during each maneuvre and used to calculate NO output. Linear regression of the NO output vs. flow was performed to derive the total maximal airway NO flux ($J'_{aw,NO}$) (nl/min) and the alveolar NO concentration ($C_{A,NO}$) (ppb) using the model of Condorelli *et al.* (19) which adjusts for possible contamination of the alveolar region by back-diffusion of NO from the airway compartment. A commercial online NO analyser system (Hypair F_{eNO}, Medisoft Cardio-respiratory instrumentation, Sorinnes, Belgium) was used for all measurements. All exhaled NO measurements were performed without a noseclip. The equation of Perillo *et al.* (36) was used to derive the rate of alveolar NO production ($V'_{A,NO}$) (nl/min) as follows:

$$V'_{A,NO} = DLNO \cdot C_{A,NO}(P_{atm} - P_{H2O}) \cdot 10^3$$

where P_{H2O} is water vapor partial pressure (assumed to be 47 mmHg) and P_{atm} is the atmospheric pressure and D_LNO is expressed in ml/min/mHg. F_{eNO} measurements were performed before the DLCO-DLNO maneuvre to avoid contamination by inspired NO in the test gas mix.

Pulmonary Blood Flow Measurements

To assess the pulmonary circulation, effective pulmonary blood flow (Q_{pulm}) was measured noninvasively by the inert-gas rebreathing

method using a commercially available device (Innocor, Innovision ApS, Glamsbjerg, Denmark). The inspired gas mixture contained 0.5% nitrous oxide (N_2O) and 0.1% sulfur hexafluoride (S_F6) as the soluble and insoluble components respectively (25). To adjust for body size, the pulmonary blood flow index (QI_{pulm}) was derived by dividing Q_{pulm} by the predicted body surface area using the formula of Mosteller (37).

Analysis

The data were not normally distributed; therefore differences between groups were assessed using the Wilcoxon rank test. Correlations as assessed by Spearman's rank coefficient were calculated to determine the strength of relationships between airway NO flux and airflow obstruction (FEV_1:VC and R_5) and alveolar NO production and effective pulmonary blood flow. Statistical analysis was performed using R software (version 3.1.1, R Foundation for Statistical Computing, Vienna, Austria).

Sample Size

Comparison of 18 children in each group allowed detection of a difference in the results equivalent to one standard deviation between the groups with 80% power at the 5% level.

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