Severe Nocturnal and Postexercise Hypoxia in Children and Adolescents with Sickle Cell Disease

Isabelle Halphen¹, Caroline Elie², Valentine Brousse⁴, Muriel Le Bourgeois⁵, Slimane Allali⁴, Damien Bonnet², Mariane de Montalembert²

1 Pediatric Emergency Department, Hospital Necker, APHP, Paris, France, 2 Paris Descartes University, Paris, France, 3 Department of Biostatistics, Hospital Necker, APHP, Paris, France, 4 Pediatrics Department and Sickle Cell Clinic, Hospital Necker, AP-HP, Paris, France, 5 Pediatric Pneumology and Allergology Department, Hospital Necker, APHP, Paris, France, 6 Pediatric Cardiology Department, M3C–Necker, AP-HP, Paris, Paris Descartes University, France

Abstract

Hypoxia is a common feature in children with sickle cell disease (SCD) that is inconsistently associated with painful crises and acute chest syndrome. To assess the prevalence and risk factors of hypoxia, we recorded daytime, nocturnal, and postexercise pulse oximetry (SpO2) values in 39 SCD patients with a median age of 10.8 years. Median daytime SpO2 was 97% (range, 89%–100%), and 36% of patients had daytime hypoxia defined as SpO2 < 96%. Median nocturnal SpO2 was 94.7% (range, 87.7%–99.5%), 50% of patients had nocturnal hypoxia defined as SpO2 < 93%, and 11(37%) patients spent more than 10% of their total sleep time with SpO2 < 90%. Median postexercise SpO2 was 94% (range, 72%–100%) and 44.7% of patients had postexercise hypoxia defined as an SpO2 decrease ≥3% after a 6-minute walk test. Among patients with normal daytime SpO2, 35% had nocturnal and 42% postexercise hypoxia. Compared to 9 patients without daytime, nocturnal, or postexercise hypoxia, 25 patients with hypoxia under at least one of these three conditions had greater anemia severity (P = 0.01), lower HbF levels (P = 0.04), and higher aspartate aminotransferase levels (P = 0.03). Males predominated among patients with postexercise hypoxia (P = 0.004). Hypoxia correlated neither with painful crises nor with acute chest syndrome. Of 32 evaluable patients, 6 (18.8%) had a tricuspid regurgitation velocity ≥2.6 m/s, and this feature was associated with anemia (P = 0.044). Median percentage of the predicted distance covered during a 6-minute walk test was 86% [46–120]; the distance was negatively associated with LDH (P = 0.044) and with a past history of acute chest syndrome (P = 0.009). In conclusion, severe episodes of nocturnal and postexercise hypoxia are common in children with SCD, even those with normal daytime SpO2.

Introduction

Sickle cell disease (SCD) is associated with chronic hemolysis, resistance to nitric oxide (NO) bioactivity, small-vessel obstruction, and ischemia-reperfusion injury. Respiratory complications such as acute chest syndrome (ACS) and pulmonary hypertension (PH) are the most common identifiable causes of premature death in adults with SCD [1]. Many studies of children with SCD showed hypoxia during the day, while sleeping, or after exercising [2–11]. The consequences of hypoxia in SCD children are unclear. In some studies, hypoxia was associated with pain [7] and ACS [11], but other studies failed to replicate these findings [3], [8]. Associations with neurological complications have been reported [9], [10]. While hypoxia in children with SCD was associated with elevation of the tricuspid regurgitation velocity (TRV) [4], [5], [12], there is still intense controversy over the indiscriminate use of the TRV to estimate pulmonary pressure in this condition. TRV appears to have limitations in specificity, but may indicate the presence of PH, which must be confirmed by right heart catheterization [13], [14]. None of the available studies in children with SCD simultaneously evaluated daytime, nocturnal, and postexercise oxygen saturations, together with tonsil size, painful events, lung function, echocardiographic data including TRV, blood markers for hemolysis, and performance on the 6-minute walk test (6 MWT). The 6 MWT has shown good reliability and is increasingly used in children affected with chronic disease to evaluate their sub-maximal functional exercise capacity [15].

Here, our primary objective was to assess the prevalence of hypoxia in children with SCD by performing, not only the usual daytime measurements, but also measurements during three nights and after a 6 MWT. Secondary objectives were to identify risk factors for daytime, nocturnal, and postexercise hypoxia and to identify correlations linking hypoxia to vasoocclusive crises (VOCs), ACS, and TRV.

Methods

Study Design

We conducted a prospective single-center study in consecutive children with SCD who had either the homozygous SS or the S/β⁰ thalassemia genotype and who were seen at the SCD clinic of the Necker Hospital, Paris, France. Our local institutional review board (Conseil Ethique Necker-Enfants Malades) approved the study. Written informed consent was obtained for all patients from the
parents/guardians, and from the minors who were old enough to understand. The data used in this study were anonymized.

The SCD phenotype was confirmed by hemoglobin (Hb) electrophoresis and/or high-performance liquid chromatography. Patients who were in a chronic transfusion program were excluded. All investigations were performed in patients in stable condition, defined as a 3-month period without admission for painful events and without blood transfusion therapy.

We included 42 consecutive patients. Three patients were excluded because their echocardiograms showed asymptomatic congenital heart disease (pulmonary valve stenosis in 2 and persistent arterial duct in 1). Among them, 38 had the SS genotype and one had 5/β0 thalassemia.

Clinical and Laboratory Data

VOCs were defined as painful episodes having no other explanation than vasocclusion and requiring therapy prescribed by a healthcare professional in a medical setting such as a hospital, clinic, or emergency room. The number of VOCs during the year before inclusion was determined retrospectively by hospital chart review. The number of VOCs during the year after inclusion was made determined prospectively by having the patients and/or their parents complete a standardized form; however, few patients/parents completed the forms regularly and we therefore used the number of VOCs recorded in the hospital charts. We also reviewed the hospital charts for data on the history of ACS (defined as a new pulmonary infiltration combined with one or more of the following: fever, cough, sputum production, tachypnea, dyspnea, and new-onset hypoxia). Hydroxyurea treatment was recorded. Tonsil size and blood pressure were measured prospectively. The day when the lung function tests were performed, we measured Hb; reticulocyte, leukocyte, and platelet counts; and the fetal Hb level (HbF, determined using the HPLC system from Bio-Rad). As markers for hemolysis, we recorded the serum levels of aspartate aminotransferase (AST), total bilirubin, and lactate dehydrogenase (LDH), determined using standard methods.

Oxygen saturation (SpO2) was measured by finger pulse oximetry at rest using a MasimoRadical SET pulse oximeter (Masimo, Irvine, CA). We defined daytime hypoxia as SpO2<96% [11]. Nocturnal finger pulse oximetry was recorded using a Nonin device (Nonin Medical, Plymouth, MN) during three consecutive nights, at home, in 30 patients. We considered the median nighttime SpO2 over the three nights and the worst mean SpO2 during any of the three nights. We defined nocturnal hypoxia as SpO2<93% [16]. We also recorded the percentage of sleep time spent with SpO2<90%.

An unencouraged 6 MWT was conducted as recommended by the American Thoracic Society Pulmonary Function Standards Committee [17]. The 6 MWT was performed on a 20-m straight track. Patients were instructed to cover the largest possible distance in 6 minutes as follows: “the objective of this test is to walk as quickly as you can during 6 minutes. You may slow down or stop if you need to, and restart the walk as soon as you can. I will announce every minute that goes by. Your goal is to walk as fast as you can for 6 minutes. I will write down the distance you walked.” Turns were made on both ends of the 20-m track. The distance was recorded with a lap counter. At the end of the test, patients were asked to stand still, and the distance covered in the final partial lap was measured. The total distance covered was calculated by multiplying the number of laps (back and forth once) by 40 m then adding the final lap. The total distance walked was rounded off to the nearest meter. We expressed the 6 MWT distances as percentages of the predicted distance for age, using the reference values reported by Geiger et al. in 2007 [18]. We defined postexercise hypoxia as an SpO2 decline ≥3% versus baseline [5]. The postexercise SpO2 recording failed in 1 patient.

Echocardiography

Echocardiographic data were acquired from standard parasternal and apical views. The left ventricular mass index was calculated using the American Society of Echocardiography. Right atrial pressure (RAP) was defined as normal (3 mmHg) if respiratory changes in inferior vena cava diameter were ≥0.40 mm. Right ventricular systolic pressure was computed as 4 · TRV2+RAP. Mean pulmonary artery pressure was computed as 4 · protodiastolic velocity of pulmonary regurgitation2+7 mmHg. Diastolic pulmonary artery pressure was computed as 4 · telodiastolic velocity of pulmonary regurgitation2+7 mmHg.

Lung Function Tests

Spirometry, plethysmography, and measurement of lung diffusion capacity for carbon monoxide (DLCO) were performed in all children as recommended by the American Thoracic Society/European Respiratory Society. DLCO was adjusted for β2-concentration. We recorded forced expiratory volume in 1 second (FEV), forced vital capacity (FVC), and FEV/FVC; and we classified the ventilation phenotypes as normal, restrictive, obstructive, or mixed according to guidelines issued by the American Thoracic Society/European Respiratory Society [19].

Data Analysis

For between-group comparisons, we used Fisher’s exact test for categorical variables and Wilcoxon test’s for continuous variables. Relations between quantitative variables were assessed using Spearman’s correlation coefficient. P values<0.05 were considered significant. Statistical analysis was performed with R software (http://cran-project.org).

Results

Clinical and Laboratory Features

Table 1 and Table 2 list the main features of the 39 study patients. None had clinical symptoms suggesting obstructive sleep apnea.

Lung and Heart Function

Lung function tests were abnormal in 15 (38.5%) patients, of whom 4 had an obstructive pattern, 8 a restrictive pattern, and 3 a mixed pattern. Hb-adjusted DLCO was normal in all but 2 patients, whose values were 69% and 72% of predicted, respectively.

Of the 38 patients who underwent echocardiography, 7 had mild left ventricular dilatation (Z score>2). All patients had normal left ventricular ejection fraction values (>60%). TRV was measurable in 32/38 patients and was ≥2.6 m/s in 6 (19%). Protodiastolic pulmonary regurgitation velocity (estimated mean pulmonary artery pressure) was measurable in 19/38 patients, none of whom had values greater than 2.0 m/s. Of 16/38 patients with whom telediastolic pulmonary regurgitation velocity (estimated diastolic pulmonary artery pressure) was measurable, none had values greater than 1.4 m/s. Overall, systolic, mean, and/or
diastolic pulmonary artery pressures were assessable in 35/38 patients, none of whom had elevated values.

Prevalence of Hypoxia

**Day.** Of the 39 patients, 14 (36%) had SpO2 values lower than 96% during the day. Median daytime SpO2 was 97% (range, 95%–100%).

**Night.** Of the 30 patients with nocturnal SpO2 recordings, 15 (50%) had SpO2 values <93%. The median nighttime oxygen saturation was 94.7% (range, 87.7–99.5%). Of the 30 patients, 11 (37%) spent more than 10% of their total sleep time with SpO2 <90%.

**Postexercise.** Of the 38 patients with post-6 MWT data, 25 (65%) had SpO2 < 96% and 10 (26%) had SpO2 < 90%. Median postexercise SpO2 was 94% (range, 72%–100%). Compared to baseline, the SpO2 decline after the 6 MWT was ≥3% in 17 (44.7%) patients.

**Associations across Daytime, Nocturnal, and Postexercise SpO2 Values**

Interpretable SpO2 recordings were available in all three conditions for 34 patients.

A significant association was found between daytime and nocturnal SpO2 values (P = 0.02). Among children with normal daytime SpO2 values, 35% had nocturnal hypoxia (compared to 80% of those with daytime hypoxia). Daytime and postexercise SpO2 values were not significantly associated with each other (P = 0.62). The proportion of children with a postexercise SpO2 decline ≥3% was 42% in the group with normal daytime SpO2 and 50% in the group with daytime hypoxia. We found no significant association between nocturnal and postexercise SpO2 (P = 0.10).

**Factors Associated with Hypoxia**

Hypoxia was found under at least one of the three measurement conditions in 25 (73.5%) patients and under none of the three conditions in 9 patients. Table 3 compares these two groups. Hypoxia was associated with greater anemia severity (P = 0.01), lower HbF (P = 0.04), and higher AST (P = 0.03). Hypoxia was associated neither with a past history of ACS nor with the number

---

### Table 1. Main characteristics of the 39 children and adolescents with sickle cell disease (part 1).

|                  | N  | %   |
|------------------|----|-----|
| Male gender      | 14 | 36  |
| Enlarged tonsils | 16/37 | 43  |
| HC treatment     | 9  | 23  |
| History of ACS   | 15 | 38.5|

doi:10.1371/journal.pone.0097462.t001

### Table 2. Main characteristics of the 39 children and adolescents with sickle cell disease (part 2).

|                           | Median | Range  | Normal range |
|---------------------------|--------|--------|--------------|
| Age (years)               | 10.8   | 5.7–17 |              |
| BMI (Kg/m²)               | 17     | 13–24  |              |
| VOC in the past year      | 0      | 0–7    |              |
| VOC in the next year      | 0      | 0–6    |              |
| Basal heart rate (bpm)    | 97     | 75–122 |              |
| Systolic blood pressure (mmHg) | 108     | 87–132 |              |
| Diastolic blood pressure (mmHg) | 65      | 47–85  |              |
| Hemoglobin (g/dL)         | 7.9    | 5.2–10.6|              |
| Leukocytes (Giga/L)       | 10.8   | 5.7–21.5|              |
| Reticulocytes (Giga/L)    | 239    | 43–443 |              |
| Fetal hemoglobin (%)      | 9.2    | 0.8–28 |              |
| Platelets (Giga/L)        | 379    | 118–742|              |
| Aspartate aminotransferase (IU/L) | 62      | 35–132 | 9–40         |
| Alanine aminotransferase (IU/L) | 24      | 11–68  | 7–40         |
| Total bilirubin (µmol/L)  | 42     | 13–163 | 0–17         |
| Lactate dehydrogenase (IU/L) | 1421   | 618–1893| 125–243     |
| Creatinine (µmol/L)       | 36     | 20–57  | 20–75        |
| 6 MWT distance (% predicted distance) | 86 | 46–120 |              |

HC, hydroxycarbamide; ACS, acute chest syndrome; BMI, body mass index; VOC, vasoocclusive crisis.

doi:10.1371/journal.pone.0097462.t002
of VOCs. Hypoxia was less common in hydroxyurea-treated patients, but the difference was not statistically significant (P = 0.31).

Lower values for the lowest nocturnal SpO2 (Table 4) were associated with greater anemia severity (P = 0.006), lower HbF levels (P = 0.01), abnormal lung function tests (P = 0.003), daytime SpO2 (P = 0.03), and postexercise SpO2 (P = 0.04) but not with larger tonsil size, number of VOCs, or history of ACS. The worst nocturnal SpO2 value was strongly associated with the median nocturnal SpO2 (P = 10^-5) and with the percentage of sleep time spent with SpO2 < 90% (P = 0.0007).

We compared the 17 patients with and the 21 patients without a postexercise SpO2 decline ≥ 3% (Table 5). Factors significantly associated with a decline ≥ 3% were male gender (P = 0.004) and a higher percentage of sleep time spent with SpO2 < 90% (P = 0.04).

Factors Associated with Elevated Tricuspid Regurgitation Velocity (TRV)

TRV elevation to ≥ 2.60 m/sec was found in six (18.8%) of 32 children in whom it could be measured. Importantly, the 4 patients with TRV > 2.6 m/s had normal estimated mean and/or diastolic pulmonary artery pressures.

TRV ≥ 2.60 m/sec was associated with greater anemia severity (8.2 g/dL [range, 6.5–10.6] versus 7.4 [range, 6.4–8.1], P = 0.045). In contrast, TRV ≥ 2.60 m/sec was not associated with any of the other study parameters, including hypoxia under any of the three measurement conditions (data not shown).

6-Minute Walking Test (6 MWT)

Median distance walked during the 6 MWT was 547 m (range, 303–702 m). The median percentage of predicted distance was 86% [46–120] (Figure 1). The 6 MWT distance correlated negatively with the LDH levels (P = 0.044, rho = –0.35). It was also strongly associated with a past history of ACS: median, 94% [50–120] in children with no past history of ACS and 83% [46–95] in those with at least one ACS episode (P = 0.009).

Discussion

We used pulse oximetry to assess the prevalence of hypoxia in children and adolescents with SCD during the day, at night during sleep, and after exercise. We found that 36% of patients had hypoxia under steady-state daytime conditions, 50% at night while sleeping, and 45% after a 6 MWT. These results were observed in children who did not have the most severe forms of the disease as, in our unit, such children receive chronic monthly blood transfusions, which was an exclusion criterion for the present study.

Many studies used pulse oximetry to assess hypoxia in children with SCD [2–11] but, to our knowledge, none obtained measurements during the day, during sleep, and after exercise in the same patients. Pulse oximetry has been shown to reliably assess oxygen saturation in patients with SCD [20], [21]. Measurement variability across three measurements obtained in the same children over a 12-month period was noted when the initial SpO2 value was ≤ 92% [22]. Whether this intraindividuall variability was related to modifications in the health condition of the patient, modifications in Hb levels, or proximity of the SpO2 value to the inflection point of the oxygen dissociation curve remained unclear.

In keeping with earlier data [3–6], factors associated with hypoxia under at least one of our three measurement conditions were greater anemia severity, lower HbF, and higher AST values (indicating greater hemolytic activity). VOC and ACS were not different between the groups with and without hypoxia under at least one of the three conditions. Importantly, normal daytime

Table 3. Risk factors for at least one type of hypoxemia (day, night, and postexercise).

| Medical history | No hypoxemia (n = 9) | Hypoxemia (n = 25) | P value |
|-----------------|----------------------|--------------------|---------|
| Age (yrs)       | 9.0 (5.7–17.0)       | 10.5 (6.2–16.8)    | 0.56    |
| Male gender     | 1 (11%)              | 11 (44%)           | 0.11    |
| BMI (Kg/m²)     | 16.3 (13.3–18.8)     | 16.1 (13.9–22.2)   | 0.33    |
| Enlarged tonsils| 4 (50%)              | 12 (48%)           | 1       |
| N of VOCs in past year | 0 (0–7) | 0 (0–2) | 0.14 |
| Hydroxyurea treatment | 3 (33%) | 10 (40%) | 1 |
| History of at least one ACS episode | 3 (33%) | 10 (40%) | 1 |
| Abnormal lung function test | 2 (22%) | 11 (44%) | 0.43 |

Laboratory tests

|                      | No hypoxemia (n = 9) | Hypoxemia (n = 25) | P value |
|----------------------|----------------------|--------------------|---------|
| Hemoglobin (g/dL)    | 8.5 (7.5–10.2)       | 7.5 (5.2–10.6)     | 0.01    |
| Leukocytes (Giga/L)  | 9.4 (5.8–16.0)       | 11.1 (5.7–21.5)    | 0.30    |
| Reticulocyte count (Giga/L) | 220 (43–276) | 246 (103–443) | 0.14 |
| Lactate dehydrogenase (IU/L) | 1126 (901–1606) | 1467 (849–1893) | 0.15 |
| Total bilirubin (µmol/L) | 41 (17–130) | 48 (22–163) | 0.60 |
| Aspartate aminotransferase (IU/L) | 49 (46–100) | 63 (39–132) | 0.03 |
| Fetal hemoglobin (%) | 11.4 (2.6–28)       | 5.7 (0.8–20.2)     | 0.04    |
| Creatinine (µmol/L)  | 37 (31–40)           | 36 (20–57)         | 0.40    |
| 6 MWT distance (% predicted distance) | 86 (46–120) | 86 (49–119) | 1 |

BMI, body mass index; VOC, vasoocclusive crisis; ACS, acute chest syndrome; 6 MWT, 6-minute walking test.
doi:10.1371/journal.pone.0097462.t003

Hypoxia in Sickle Cell Disease
SpO2 did not predict absence of nocturnal or postexercise hypoxia, although significant associations were found between these variables. Thus, among the patients with normal daytime SpO2, one-third had nocturnal hypoxia and 42% a postexercise SpO2 decline ≤3%. Nocturnal hypoxia was frequently severe: 37% of our unselected patients in stable condition spent more than 10% of their sleep time with SpO2 ≤90%. Nocturnal hypoxia was associated with greater anemia severity, low HbF, daytime SpO2, postexercise SpO2 decline, and abnormal lung function tests but not with tonsil size. In a study of 95 children (78% with the SS genotype) having a mean age of 8.2 years (range, 2.1–16.9), the percentage of sleep time spent with SpO2 ≤90% was 11.1% (range, 0–99.6), and low nocturnal SpO2 was significantly associated with a greater number of VOCs [7]. The absence of a significant association in our study between nocturnal SpO2 and number of VOCs may be ascribable to our small sample size and/or to the fact that we recorded only VOCs requiring admission.

Nocturnal hypoxia was associated with neurological complications of SCD in earlier studies [9], [10]. We did not record transcranial Doppler velocities. None of the patients experienced overt stroke before or after study enrollment.

The 6-MWT, i.e., an exercise of moderate intensity, induced a ≥3% decline in SpO2 in 45% of patients, and 26% of the overall population had postexercise SpO2 values ≤90%. Similarly, in another study, 34% of children with SCD had postexercise hypoxia [23]. Postexercise hypoxia was significantly more common in our studies in males than in females. In an earlier study, steady-state SpO2 was lower in males than in females [3].

Greater NO bioavailability and NO responsiveness has been reported in females than in males with SCD [24]. In keeping with this finding, in a study of 16 654 SCD-related deaths between 1979 and 2005 in the US, women had an older mean age at death compared to men (36.9 years [95% confidence interval, 36.5–37.4] vs. 33.4 years [33.0–33.7]) [25].

Anemia only partly explains the hypoxia seen in SCD. According to one study, anemia may explain only 5% of the arterial oxygen desaturation in children with SCD [3]. One factor that may contribute to decrease oxygen saturation in SCD is decreased affinity of the sickle Hb for oxygen related to an increased content of erythrocyte 2,3-biphosphoglycerate [26]. Chronic hemolysis may also contribute to hypoxia. Significant associations have been reported in SS children between hypoxia at rest and hemolysis, and between postexercise hypoxemia and hemorrheological abnormalities [23]. Also, reduced NO availability related to hemolysis may result in pulmonary vasculopathy responsible for ventilation-perfusion mismatching and limited oxygen uptake by Hb [27]. A correlation has been found between hypoxemia severity and markers for cellular activation (soluble L-selectin, P-selectin, VCAM-1, and leukotriene B4), suggesting a pathophysiological explanation for the onset of complications in hypoxicemic SCD patients [6]. In addition, vascular endothelial lesions may be exacerbated by reoxygenation phases, which are known to trigger inflammation [28]. This last possibility suggests a need for carefully appraising the risk/benefit ratio of sports participation in patients with SCD. Although improving cardio-

Table 4. Clinical and laboratory characteristics of patients according to lowest nocturnal SpO2.

| Medical history                  | >93% (n = 15) | ≤93% (n = 15) | P value |
|---------------------------------|--------------|--------------|--------|
| Age (yrs)                       | 10.1 (5.7–17.0) | 9.1 (6.3–16.8) | 0.65   |
| Male gender                     | 3 (20%)      | 7 (46.7%)    | 0.12   |
| BMI (Kg/m²)                     | 16.3 (13.3–19.6) | 15.7 (13.9–21.4) | 0.60   |
| Enlarged tonsils                | 6 (42.9%)    | 7 (46.7%)    | 0.84   |
| N of VOC in the past year       | 0 (0–7)      | 0 (0–2)      | 0.75   |
| Hydroxy carbamide treatment     | 4 (26.7%)    | 2 (13.3%)    | 0.65   |
| History of at least one ACS episode | 5 (33.3%) | 6 (40%)      | 0.70   |
| No abnormal lung function test  | 2 (13.3%)    | 10 (66.7%)   | 0.003  |
| SpO2 values                     |              |              |        |
| Daytime SpO2                    | 98 (89–100)  | 95 (92–99)   | 0.03   |
| Postexercise SpO2               | 97 (79–100)  | 92 (72–100)  | 0.04   |
| >10% of sleep time with SpO2 ≤90% | 1 (6.7%) | 10 (66.7%)   | 0.0007 |

| Laboratory tests                |              |              |        |
| Hemoglobin (g/dL)               | 8.4 (6.9–10.6) | 7.5 (5.2–9.5) | 0.006  |
| Leukocytes (Giga/L)             | 9.4 (5.8–16)  | 10.8 (5.7–21.5) | 0.19   |
| Reticulocyte count (Giga/L)     | 220 (43–276) | 246 (121–443) | 0.07   |
| Lactate dehydrogenase (IU/L)    | 1196 (901–1683) | 1456 (849–1893) | 0.66   |
| Total bilirubin (µmol/L)        | 36 (17–130)  | 55 (22–163)  | 0.12   |
| Aspartate aminotransferase (IU/L) | 51 (39–132) | 63 (48–93)  | 0.10   |
| Fetal hemoglobin (%)            | 12.4 (2.6–28) | 5.4 (0.8–11.7) | 0.01   |
| Creatinine (µmol/L)             | 36 (20–43)   | 36 (22–51)   | 0.91   |
| 6 MWT distance (% predicted distance) | 86 (46–120) | 87 (50–119)   | 0.66   |

BMI, body mass index; VOC, vasoocclusive crisis; ACS, acute chest syndrome; 6 MWT, 6-minute walking test.

doi:10.1371/journal.pone.0097462.t004
Pulmonary fitness is of crucial importance, additional data on potential risks related to postexercise hypoxia are needed. Of 32 patients with TRV measurements in our study, 6 (19%) had TRV values $\geq 2.6$ m/s, in keeping with reports that the prevalence of elevated TRV ranged from 11% to 30% among children with SCD [4], [29–40]. TRV $\geq 2.6$ m/s was associated with anemia but not with hemolysis; this last finding is probably ascribable to our small sample size. Of note, none of our patients had pulmonary regurgitation velocities suggesting elevated pulmonary vascular resistance. We are aware that TRV has low sensitivity as a PH screening tool in adults. Our patients did not undergo right heart catheterization and, therefore, we cannot rule out that some of them had undiagnosed PH. Of note, the patients with the highest TRV values in our study had LV dilatation. Confirming the relation between elevated TRV and LV filling pressures would require pulmonary wedge pressure or diastolic transpulmonary gradient measurement, now suggested as the reference standard tool for diagnosing postcapillary PH. TRV elevation was associated with a decrease in the 6 MWT distance in several studies of children with SCD [4], [32]. In contrast, we found no association between TRV and the 6 MWT distance. The limited distances achieved by our patients during the 6 MWT were comparable to those recently reported in children with SCD [40], [41], and in children with different chronic conditions such as juvenile idiopathic arthritis, hemophilia, spina bifida, and mildly to moderately symptomatic cystic fibrosis [42], [43]. Interestingly, Waltz et al showed that a high level of anemia, low fetal hemoglobin expression, and low red blood cell deformability independently predicted poor 6 MWT performance [41]. We confirm the association between reduced 6 MWT distances and severe hemolysis as indicated by increased LDH levels in children with SCD; as well as the negative impact of prior ACS, which to our knowledge had not been reported previously. Of note, a recent study found that a decrease in the 6 MWT distance correlated with silent infarcts in children with SCD, suggesting a role for underlying chronic hypoxia [40].

The best strategies for minimizing hypoxia-related damage are unclear. In 3 children with SCD, the introduction of hydroxycarbamide therapy was followed by the resolution of chronic Table 5. Clinical and laboratory characteristics according to the SpO2 decline induced by the 6-minute walking test.

|                          | Decline<3%(n = 21) | Decline≥3% (n = 17) | $P$ value |
|--------------------------|--------------------|---------------------|-----------|
| **Medical history**      |                    |                     |           |
| Age (yrs)                | 10.5 (5.7–17.0)    | 11.5 (6.2–15.6)     | 0.32      |
| Male gender              | 3 (14.3%)          | 10 (58.8%)          | 0.004     |
| BMI (Kg/m$^2$)           | 16.1 (13.3–24.0)   | 16.9 (14.0–21.4)    | 0.62      |
| Enlarged tonsils         | 8 (40%)            | 7 (43.8%)           | 0.82      |
| N of VOC in the past year| 0 (0–7)            | 0 (0–6)             | 0.22      |
| Hydroxyxycarbamide treatment | 6 (28.6%)    | 3 (17.6%)           | 0.48      |
| Past history of at least one ACS episode | 8 (38.1%) | 7 (41.2%) | 0.85      |
| Abnormal lung function test | 6 (28.6%)   | 8 (47.1%)           | 0.24      |
| **SpO2 values**          |                    |                     |           |
| Daytime SpO2             | 98 (89–100)        | 96 (92–100)         | 0.44      |
| Lowest nocturnal SpO2    | 95.5 (87.5–99.5)   | 92.0 (87.8–96)      | 0.051     |
| % sleep time with SpO2<90% | 0.3 (0–90.6)    | 9 (0.1–68.2)        | 0.048     |
| **Laboratory tests**     |                    |                     |           |
| Hemoglobin (g/dL)        | 8.2 (6.6–10.2)     | 7.5 (5.2–10.6)      | 0.08      |
| Leukocytes (Giga/L)      | 10.7 (5.7–16.0)    | 11.4 (7.7–21.5)     | 0.92      |
| Reticulocyte count (Giga/L) | 232 (43–443) | 239 (104–357)       | 0.90      |
| Lactate dehydrogenase (IU/L) | 1130 (618–1731) | 1467 (849–1893)    | 0.17      |
| Total bilirubin (µmol/L) | 47 (13–130)        | 39 (13–161)         | 0.55      |
| Aspartate aminotransferase (IU/L) | 56 (35–132) | 66 (39–93)         | 0.06      |
| Fetal hemoglobin (%)     | 10.1 (2.6–28)      | 7.1 (3.9–20.2)      | 0.46      |
| Creatinine (µmol/L)      | 37 (20–53)         | 35 (22–57)          | 0.20      |
| 6 MWT distance (% predicted distance) | 92 (46–120) | 86 (73–102)        | 0.71      |

BMI, body mass index; VOC, vasoocclusive crisis; ACS, acute chest syndrome; 6 MWT, 6-minute walking test.
doi:10.1371/journal.pone.0097462.t005

Hypoxia in Sickle Cell Disease

Figure 1. Distribution of the patients according to distance walked during the 6-minute walking test, expressed as % of the expected value.
doi:10.1371/journal.pone.0097462.g001
hypoxia, and this effect was not entirely ascribable to increases in Hb and HbF levels [44]. The respective indications of hydroxycarbamide, chronic blood transfusion, and hematopoietic stem cell transplantation in children with SCD and hypoxia and/or TRV elevation are not established [45], [46]. Nocturnal oxygen therapy may decrease the risk of developing hypoxemia-related vasculopathy. In a phase I controlled trial, overnight auto-adjusting positive airway pressure, with supplemental oxygen when nocturnal oxygen saturation was below 94%, improved sleep-related breathing disorders and one aspect of cognition in children with SCD, without inducing bone marrow suppression [47]. In conclusion, the prevalence of severe episodes of oxygen desaturation is high among children and adolescents with SCD. Normal daytime Spo2 values do not rule out nocturnal and/or postexercise desaturation episodes. Hypoxia is related to anemia, low HbF, and hemolysis. Many patients have a limited 6 MWT distance. The long-term consequences of hypoxia are unclear, especially regarding the risk of developing PH. Large-scale, prospective, controlled trials are needed and should include investigations of the effects of hydroxycarbamide and oxygen therapy.

Acknowledgments

We thank all patients who participated in the study, Jean-Louis Soutoul for his help in organizing nocturnal oxygen saturation recordings at home, and A Wolfe MD for her help in preparing the English version of the manuscript.

Author Contributions

Conceived and designed the experiments: IH CE VB MLB SA. Analyzed the data: IH CE VB MLB SA DB. Performed the lung function tests: IH MLB. Analyzed the cardiac data: DB.

References

1. Miller CA, Gladwin MT (2011) Pulmonary complications of sickle cell disease. Am J Respir Crit Care Med 183: 1145–1165.
2. Needelman JP, Franco ME, Varlotta L, Reber-Brodecki D, Bauer N, et al. (1999) Mechanisms of nocturnal oxyhemoglobin desaturation in children and adolescents with sickle cell disease. Pediatr Pulmonol 28: 418–422.
3. Quinn CT, Ahmad N (2005) Clinical correlates of steady-state oxyhemoglobin desaturation in children who have sickle cell disease. Br J Haematol 129: 129–134.
4. Minniti C, Sable C, Campbell A, Rana S, Ensing G, et al. (2009) Elevated tricuspid regurgitant jet velocity in children and adolescents with sickle cell disease: association with hemolysis and hemoglobin oxygen desaturation. Haematologica 94: 340–347.
5. Campbell A, Minniti C, Nouria M, Artea M, Rana S, et al. (2009) Prospective evaluation of hemoglobin oxygen saturation at rest and after exercise in pediatric sickle cell disease patients. Br J Haematol 147: 332–339.
6. Setty BNY, Stuart MJ, Dampier C, Broddeki D, Allen JL, et al. (2003) Hypoxaemia in sickle cell disease: biomarker modulation and relevance to pathophysiology. Lancet 362: 1450–1455.
7. Hargrave DR, Wade A, Evans JP, Hevesi D, Kirmidh F (2003) Nocturnal oxygen saturation and painful sickle cell crises in children. Blood 101: 846–848.
8. Uuong EC, Boyd JH, DeBaun MR (2006) Daytime pulse oximeter measurements do not predict incidence of pain and acute chest syndrome episodes in sickle cell anemia. J Pediatr 149: 707–709.
9. Kirmidh F, Hevesi D, Kermelg P, Wade A, Lane R, et al. (2001) Nocturnal hypoxaemia and central-nervous-system events in sickle-cell disease. Lancet 357: 1635–1639.
10. Cox SE, Makani J, Newton CR, Prentice AM, Kirkham FJ (2013) Mortality rates and age at death from sickle cell disease: U.S., 1979–2005. Public Health Rep 128: 110–116.
11. Rackoff WR, Kunkel N, Silber JH, Asakura T, Ohene-Frempong K (1993) Pulse oximetry in sickle cell disease. J Pediatr 149: 707–709.
12. Pashankar FD, Carbonella J, Bazzy-Asaad A, Friedman A (2008) Prevalence and risk factors of elevated pulmonary artery pressures in children with sickle cell disease. Pediatr 121: 777–782.
13. Gladwin MT, Sachev V, Jenson ML, Shinzuka Y, Pehin JF, et al. (2004) Pulmonary hypertension as a risk factor for death in patients with sickle cell disease. N Engl J Med 350: 806–895.
14. Parent F, Bachur D, Inam J, Liourent D, Dris F, et al. (2011) A hemodynamic study of pulmonary hypertension in sickle cell disease. N Engl J Med 365: 44–55.
15. Hassan J, van der Net J, Holders PJM, Prakken BJ, Takkon T (2010) Six-minute walk test in children with chronic conditions. Br J Sports Med 44: 270–274.
16. American Thoracic Society (1996) Standards and indications for cardiopulmonary sleep studies in children. Am J Respir Crit Care Med 155: 866–871.
17. ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories (2002) ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med 166: 111–117.
18. Gerirer R, Strazak A, Trembl B, Gasser K, Kleinmasser A, et al. (2007) Six-minute walk test in children and adolescents. J Pediatr 150: 395–399.
19. Pellegrino R, Vigie G, Brusasco V, Crapo RO, Burgos F, et al. (2005) Interpretative strategies for lung function tests. Eur Respir J 26: 948–968.
20. Ortiz FO, Aldrich TK, Nagel RL, Benjamin Lj (1999) Accuracy of pulse oximetry in sickle cell disease. Am J Respir Crit Care Med 159: 447–451.
21. Fitzgerald RR, Johnson A (2001) Pulse oximetry in sickle cell anemia. Crit Care Med 29: 1085–1086.
22. Mullin JE, Cooper B, Seecan S, Strunk R, Rosen C, et al. (2010) Variability of pulmonary oximetry measurement over 1 year in children with sickle cell disease depends on initial oxygen saturation measurement. Pediatr Blood Cancer 24: 1017–1019.
23. Waltz X, Romana M, Lalanne-Mistril MH, Machado RF, Lamarr Y, et al. (2013) Hemoglobin-oxygen-induced hemoglobin oxygen desaturation in children with sickle cell disease. Haematologica 98: 1039–1044.
24. Gladwin MT, Schechter AN, Ogwubele FP, Coles WA, Kreit D, et al. (2003) Divergent nitric oxide bioavailability in men and women with sickle cell disease. Circulation 107: 271–276.
25. Lanzkron S, Carroll CP, Haywood C, et al. (2013) Mortalitir rates and age at death from sickle cell disease: U.S., 1979–2005. Public Health Rep 128: 110–116.
26. Miller PJ (1974) Oxygen transport in sickle cell anemia. Am Intern Med 133: 565–579.
27. Kato GJ, Gladwin MT, Steinberg MH (2007) Deconstructing sickle cell disease: reappraisal of the role of hemoglobin in the development of clinical subphenotypes. Blood Reviews 21: 37–47.
28. Kaul DK, Hebbel RP (2000) Hypoxia/reoxygenation causes inflammatory response in transgenic sickle mice but not in normal mice. J Clin Invest 106: 411–420.
29. Ambru++o SJ, Gunawardena S, Sakara A, Windsor B, Lundford L, et al. (2006) Elevation of tricuspid regurgitant jet velocity, a marker for pulmonary hypertension in children with sickle cell disease. Pediatr Blood Cancer 47: 907–913.
30. Colombatti R, Maschietto N, Varotto E, Grison A, Grazzina N, et al. (2010) Pulmonary hypertension in sickle cell disease under 10 years of age. Br J Haematol 150: 661–669.
31. Gordeuk VR, Minniti CP, Nmouria M, Campbell AD, Gregory Ensing S, et al. (2011) Elevated tricuspid regurgitation velocity and decline in exercise capacity over 22 months of follow up in children and adolescents with sickle cell anemia. Haematologica 96: 33–40.
32. Chaudry RA, Cakes M, Karu T, Hutchinson C, Ball S, et al. (2011) Paediatric sickle cell disease: pulmonary hypertension but normal vascular resistance. Arch Dis Child 96: 131–136.
33. Hagar RW, Michlisch JG, Gardner J, Vichinsky EP, Morris CR (2008) Clinical differences between children and adults with pulmonary hypertension and sickle cell disease. Br J Haematol 140: 104–112.
34. Onyekwere OG, Campbell A, Teshome M, Onyeagoro S, Sylvan C, et al. (2007) Pulmonary hypertension in children and adolescents with sickle cell disease. Pediatr Cardiol 29: 309–312.
35. Sachdev V, Kato GJ, Gibbs JS, Barst R, Machado RF, et al. (2011) Echocardiographic markers of elevated pulmonary pressure and left ventricular diastolic dysfunction are associated with exercise intolerance in adults and children with homoyzygous sickle cell anemia in the United States and United Kingdom. Circulation 124: 1452–1460.
36. Dahoui HA, Hayek MN, Nteetr P, Arabi MT, Muwakitt SA, et al. (2010) Pulmonary hypertension in children and young adults with sickle cell disease: evidence for familial clustering. Pediatr Blood Cancer 54: 389–402.
37. Lee MT, Snall T, Khan MA, Rosenzweig ER, Barst R, et al. (2009) Doppler defined pulmonary hypertension and the risk for death in children with sickle cell disease. Br J Haematol 146: 437–441.
38. Nelson SC, Adade BB, McDonough EA, Moquist KL, Hennessy JM (2007) High prevalence of pulmonary hypertension in children with sickle cell disease. J Pediatr Hemato/Oncol 29: 334–337.
39. Liem R1, Nevin MA, Prestridge A, Young LT, Thompson AA (2009) Tricuspid regurgitant jet velocity elevation and its relationship to lung function in pediatric sickle cell disease. Pediatr Pulmonol 44: 281–289.

40. Chapusette R, Dedeken L, Le PQ, Heijmans C, Decleek C, et al. (2013) Reduction of the six-minute walk distance in children with sickle cell disease is correlated with silent infarct: results from a cross-sectional evaluation in a single center in Belgium. Blood 122: abstract 2107.

41. Waltz X, Romana M, Hardy-Dessources MD, Lamarre Y, Divialle-Doumdo L, et al. (2013) Hematological and hemorrheological determinants of the six-minute walk test performance in children with sickle cell anemia. PLoS One 8: e77830.doi:10.1371/journal.pone.007830.

42. Gulmans VA, van Veldhoven NH, de Meer K, Holders PJ (1996) The six-minute walking test in children with cystic fibrosis: reliability and validity. Pediatr Pulmonol 22: 85–89.

43. Pereira FM, Ribeiro MA, Ribeiro AF, Toro AA, Ribeiro JD (2011) Functional performance on the six-minute walk test in patients with cystic fibrosis. J Bras Pneumol 37: 735–744.

44. Singh SA, Koumbourlis AC, Aygun B (2008) Resolution of chronic hypoxemia in pediatric sickle cell patients after treatment with hydroxyurea. Pediatr Blood Cancer 50: 1258–1260.

45. Pashankar FD, Carbonella J, Bazzy-Asaad A, Friedman A (2008) Longitudinal follow-up of elevated pulmonary artery pressure in children with sickle cell disease. Br J Haematol 144: 736–741.

46. Colombatti R, Varotto E, Ricato S, Nardo D, Maichietto N, et al. (2011) Tricuspid regurgitant velocity elevation in a three-year old child with sickle cell anemia and recurrent acute chest syndromes reversed not by hydroxyurea but by bone marrow transplantation. Hematol Rep 3 :e12.doi:10.4081/hr.2011.e12.

47. Marshall MJ, Bucks RS, Hogan AM, Hambleton IR, Height SE, et al. (2009) Auto-adjusting continuous positive airway pressure in children with sickle cell anemia: results of a phase I randomized controlled trial. Haematologica94: 1006–1010.