Prospective evaluation of haemoglobin oxygen saturation at rest and after exercise in paediatric sickle cell disease patients

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Summary

Low steady state haemoglobin oxygen saturation in patients with sickle cell anaemia has been associated with the degree of anaemia and haemolysis. How much pulmonary dysfunction contributes to low saturation is not clear. In a prospective study of children and adolescents with sickle cell disease aged 3–20 years at steady state and matched controls, 52% of 391 patients *versus* 24% of 63 controls had steady state oxygen saturation <99% ($P < 0.0001$), 9% of patients *versus* no controls had saturation <95% ($P = 0.008$) and 8% of patients *versus* no controls had exercise-induced reduction in saturation ≥3%. Decreasing haemoglobin concentration ($P ≤ 0.001$) and increasing haemolysis ($P ≤ 0.003$) but not pulmonary function tests were independent predictors of both lower steady-state saturation and exercise-induced reduction in saturation. Neither history of stroke nor history of acute chest syndrome was significantly associated with lower steady-state oxygen saturation or exercise-induced reduction in saturation. Tricuspid regurgitation velocity was higher in patients with lower steady state haemoglobin oxygen saturation ($P = 0.003$) and with greater decline in oxygen saturation during the six-minute walk ($P = 0.022$). In conclusion, lower haemoglobin oxygen saturation is independently associated with increasing degrees of anaemia and haemolysis but not pulmonary function abnormalities among children and adolescents with sickle cell disease.

Keywords

sickle cell disease; paediatric; oxygen saturation; six-minute walk; pulmonary hypertension

A lowering of the haemoglobin oxygen saturation as determined by pulse oximetry is common in patients with sickle cell disease at steady state despite the lack of overt evidence of pulmonary dysfunction or intrinsic lung disease (Rackoff *et al*, 1993; Homi *et al*, 1997; Setty *et al*,

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2003; Quinn & Ahmad, 2005). Decreased affinity of haemoglobin S for oxygen (Seakins *et al*, 1973; Ueda *et al*, 1979) and increased erythrocyte 2,3-bisphosphoglycerate (Milner, 1974) may partially, but probably not fully, explain decreased oxygen saturation in sickle cell disease given the wide range of saturations in patients. Several groups have reported the association of low with elevated serum lactate dehydrogenase, severity of anaemia and/or reticulocytosis, suggesting a possible aetiological relationship of haemolysis with hypoxemia (Rackoff *et al*, 1993; Homi *et al*, 1997; Setty *et al*, 2003; Quinn & Ahmad, 2005; Kato *et al*, 2006). It has been proposed that this association might involve haemolysis-associated ventilation-perfusion mismatch (Kato *et al*, 2007a).

A developing paradigm of sickle cell disease manifestations proposes two overlapping but distinctively characteristic subphenotypes (Kato *et al*, 2007a). In the vasculopathy subphenotype, complications such as stroke, pulmonary hypertension, priapism and leg ulcers predominate and are linked to the degree of haemolysis and associated vascular dysfunction. In the vaso-occlusive subphenotype, markers of increased blood viscosity predict risk for infarctive complications, such as frequent pain crises, acute chest syndrome and avascular necrosis of bone. Steady state haemoglobin oxygen desaturation is a risk factor for overt stroke (Quinn & Sargent, 2008) and for pulmonary hypertension (Pashankar *et al*, 2008; Liem *et al*, 2009; Minniti *et al*, 2009) in sickle cell disease, two of the prominent manifestations of the haemolytic subphenotype. Nocturnal oxygen desaturation is also a risk factor for stroke, silent infarct or seizure (Kirkham *et al*, 2001). On the other hand, reported associations of lower haemoglobin oxygen saturation with increased episodes of pain (Hargrave *et al*, 2003) and acute chest syndrome (Rackoff *et al*, 1993), manifestations of the vaso-occlusive subphenotype, have not been confirmed in other studies (Quinn & Ahmad, 2005; Uong *et al*, 2006).

We recently reported the initial results of an ongoing prospective, multicenter study of the prevalence and risk factors for developing pulmonary hypertension in children with sickle cell disease (PUSH Study) (Minniti *et al*, 2009). Both haemolysis and oxygen desaturation were independently associated with elevated tricuspid regurgitation velocity in this study. Pulmonary function testing and a six-minute walk were performed as a part of this study and pulse oximetry measurements were made before and after the walk. We hypothesized that lower oxygen saturations at steady state and oxygen desaturation during the six-minute walk may reflect pulmonary ventilation/perfusion mismatch related to the vascular effects of accelerated haemolysis. We investigated the relationship of oxygen saturation with medical history, echocardiographic measurements, pulmonary function testing and laboratory test results in this cohort.

Methods

Enrollment of participants

A total of 391 patients with sickle cell disease and 63 controls were recruited at three tertiary medical centers: Children's National Medical Center and Howard University Hospital in Washington DC, and University of Michigan, in Ann Arbor, Michigan. Of these, 310 patients and 54 controls were included in the initial report (Minniti *et al*, 2009). The patients were of 3–20 years of age and had a diagnosis of sickle cell disease confirmed with haemoglobin electrophoresis or high performance liquid chromatography. All phenotypes were included, such as haemoglobins SS, SC, Sb-thalassemia, SD, SO-Arab and others. The controls were matched by age, sex and ethnicity to every sixth patient with sickle cell disease. Children with sickle cell trait ($n = 17$) and haemoglobin C trait ($n = 5$) also qualified as controls. Patients were invited to participate on a consecutive basis, as they presented for routine outpatient care; no attempt was made to select them by known or perceived risk factors. To ensure that patients were enrolled at steady state, at least 3 weeks had to have elapsed since hospitalization,

emergency department or clinic visit for acute chest syndrome, pain crisis, infection or other sickle cell disease-related complication. Written informed consent was obtained for all participants. Parents or legal guardians provided consent for children ages 3–17 years. Participants from ages 18 to 20 years provided consent for themselves. Participants from 7 to 17 years provided additional written informed assent. Patients receiving regular blood transfusions or hydroxycarbamide therapy were not excluded.

Clinical evaluation

Patients underwent medical history, physical examination and measurement of oxygen saturation by pulse oximetry. Venous blood was drawn for complete blood count, reticulocyte count and serum chemistries.

Six-minute walk test

An unencouraged six-minute walk test was conducted in a predetermined course, at least six feet wide, as per the guidelines of the American Thoracic Society (ATS) (ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories 2002). This test measures the distance that an individual can quickly walk on a flat, hard surface in a period of 6 min. It is self-paced and rest is allowed during the exercise. The distance walked was recorded in metres for all subjects. Transcutaneous pulse oximetry haemoglobin oxygen saturation measurements were obtained before, during, and immediately after the six-minute walk test. Only children older than 5 years of age were given this test, due to the difficulty in obtaining reliable information from younger participants.

Laboratory studies

Complete blood count and reticulocyte count were performed by Beckman Coulter LH 750 Analyzer (Fullerton, CA, USA) at Howard University and the University of Michigan, and by Sysmex 2100QC (Sysmex America, Inc., Mundelein, IL, USA) at the Children's National Medical Center. Serum chemistries were performed by Beckman Coulter Unicel DXC800 at Howard University, by RXL 2 Max, Model 973626, (Dade-Behring, Inc., Dover, DE, USA) at the Children's National Medical Center and by Siemens Advia 2400 (Deerfield, IL, USA) at the University of Michigan. Pulse oximetry was measured by Criticare Model 506 Series (Waukesha, WI, USA) at Howard University, a Welch Allyn instrument (Beaverton, OR, USA) or a SureSigns VS3 No. 3000 (Philips Medical System, Andover, MA, USA) at the Children's National Medical Center and by a Masimo Rad 8 Signal Extraction Pulse Oximeter at the University of Michigan. Reticulocyte count and serum concentrations of lactate dehydrogenase, aspartate aminotransferase and total bilirubin were prospectively considered to be markers of haemolysis. Reference ranges for Howard University, Children's National Medical Center and University of Michigan, respectively, were 25–75, 42–95 and 26–104 $\times$ 10^9/l for reticulocyte count; 0–250, 141–231 and 150–300 u/l for lactate dehydrogenase; 0–50, 10–36 and 5–60 u/l for aspartate aminotransferase; 3·4–20·5, <13·7 and 1·7–17·1 µmol/l for total bilirubin.

Echocardiography

Transthoracic echocardiography was performed using the Philips Sonos 5500/7500 or iE33 (Philips Medical Systems, Best, Holland), Acuson Sequoa (Siemans Medical Systems, Mountain View, CA, USA), or General Electric VIVID 7 or VIVID I (General Electric, Milwaukee, WI, USA). All images were recorded digitally and subsequently reviewed on an offline digital work station. Cardiac images were obtained, measurements were performed, and the studies were interpreted according to the guidelines of the American Society of Echocardiography (Lai *et al*, 2006). Tricuspid regurgitation was assessed in the parasternal long and short-axis, and apical four-chamber views. Continuous-wave Doppler sampling was

used to determine the peak tricuspid regurgitation velocity, which reflects systolic pulmonary artery pressure (Berger *et al*, 1985). Left ventricular diastolic function was assessed by measuring the tissue Doppler E wave at the basilar segments of the left ventricular free wall and the interventricular septum. The mitral inflow E wave to tissue Doppler E wave (E/Etdi) ratio reflects left atrial and left ventricular filling pressures (Nagueh *et al*, 1997). Based on the systemic blood pressure-and age-adjusted mean + 2 SDs in the controls of this study, a peak tricuspid regurgitation velocity of $\geq 2\cdot 60$ m/s and an E/Etdi $\geq 8\cdot 9$ was considered to be elevated.

Pulmonary function testing

Participants underwent spirometry and plethysmography, according to the ATS standard protocols (Macintyre *et al*, 2005; Miller *et al*, 2005; Wanger *et al*, 2005). Pulmonary function test results were expressed as a percentage of the predicted value based on ethnic appropriate reference standards for spirometry (Hankinson *et al*, 1999). For plethysmography, results were expressed as a percentage of the predicted race-adjusted reference standards (Pellegrino *et al*, 2005). The pulmonary function testing systems used were VMAX 62J (Sensomedics, Vienna, Austria) at the University of Michigan and Howard University and Elite DX (Medical Graphics, St. Paul, MN, USA) at the Children's National Medical Center. The following parameters were included in the analysis: forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1), FEV1/FVC, total lung capacity (TLC).

Statistical analysis

Analysis of variance was used to compare continuous variables among groups of patients according to different baseline haemoglobin oxygen saturations. The *post hoc* Bonferroni test was used for pair-wise comparisons. The Student's *t*-test or the Kolmogorov–Smirnov nonparametric test (whichever appropriate) was used to compare continuous variables between cases and controls, or two groups of patients according to six-minute walk desaturation. For categorical variables we applied the chi-square test. A stepwise linear regression model was developed to identify independent predictors of steady-state haemoglobin oxygen saturation. A stepwise logistic regression model was developed to determine independent predictors of a decline in oxygen saturation of three points or more during the six-minute walk. *P* values <0·05 were considered significant. Analyses were performed with STATA 10 (StataCorp, College Station, TX, USA).

Results

Steady-state haemoglobin oxygen saturation

The distribution of steady state haemoglobin oxygen saturation in 391 children with sickle cell disease and 63 controls is depicted in Fig 1. Two hundred and two (52%) of the patients with sickle cell disease compared to 15 (24%) of the controls had haemoglobin oxygen saturation <99% (*P* < 0·0001) while 9% of patients *versus* no controls had saturation <95% (*P* = 0·008). Table I presents clinical findings stratified according to those that might be predictors of steady state oxygen saturation level and those that could be viewed as outcomes associated with the degree of saturation. In bivariate analysis of potential predictors of oxygen saturation, the following showed significant correlations with lower saturation: male sex, severe sickling phenotype, lower values for weight-for-age *z*-score, haemoglobin concentration and TLC, and higher values for four markers of haemolysis and a haemolytic component derived from these markers. In bivariate analysis of potential outcomes, lower oxygen saturation was associated with higher values for tricuspid regurgitation velocity, which reflects systolic pulmonary artery pressure, and with more frequently having an exercise-induced decline in haemoglobin oxygen saturation. Lower oxygen saturation was not associated with increasing mitral valve E/Etdi, which reflects left ventricular diastolic dysfunction, nor with shorter distance walked in the six-minute walk or histories of acute chest syndrome, frequent pain episodes or stroke.

Independent predictors of low baseline oxygen saturation by multiple linear regression analysis

Both decreasing haemoglobin concentration ($P = 0.001$) and increasing degree of haemolysis ($P < 0.0001$) were independent predictors of lower steady state oxygen saturation among the patients overall and age was also a predictor ($P = 0.043$) (Table II). TLC did not have an independent association with lower oxygen saturation. According to this model and with other variables held constant, an increase in the haemolytic index of 2 standard deviations (SD) was associated with a decrease in the oxygen saturation of 1·4% (95% confidence interval of 0·9–1·9%). Likewise, a reduction in the haemoglobin concentration of 40 g/l is associated with a decrease in the oxygen saturation of 1·0% (0·4–1·6%). FEV1, FVC, FEV1/FVC ratio and TLC did not have independent associations with oxygen saturation.

Decline in haemoglobin oxygen desaturation during the six-minute walk test (Table III)

Oxygen saturation was measured before and after the six-minute walk test. A cut-off point of 3% or greater decline in saturation during the walk was used to categorize the patients into two groups. Among 323 patients with this available data, 27 (8%) had a reduction in oxygen saturation of three points or more during the six-minute walk. None of the controls had such a decline during the six-minute walk. In bivariate analyses, younger age, severe sickling phenotype, lower values for weight-for-age z-score, haemoglobin concentration and baseline oxygen saturation and higher values for the haemolytic component were predictors of exercise-induced decline in oxygen saturation of three points or more. Histories of asthma and obstructive sleep apnea did not predict desaturation during exercise. Of potential outcomes, exercise-induced decline in oxygen saturation had a significant association with higher tricuspid regurgitation velocity but not with history of acute chest syndrome or frequent severe pain episodes. Interestingly, exercise-induced decline in oxygen saturation appeared to be a better predictor of elevated tricuspid regurgitation velocity than steady-state saturation: 29% of patients with a decline in saturation of three points or more had elevated tricuspid regurgitation velocity compared to 17% of patients with steady state saturation <95%.

Independent predictors of exercise-induced decrease in oxygen saturation by logistic regression analysis

By multivariate analysis, lower haemoglobin concentration ($P = 0.0003$) and greater degree of haemolysis ($P = 0.003$) were each independently associated with increased odds of exercise-induced decrease in oxygen saturation (Table IV). Age, height, sex, history of acute chest syndrome, baseline oxygen saturation and histories of asthma or sleep apnea were not significantly related to oxygen desaturation during the six-minute walk test. Pulmonary function tests were not independent predictors of exercise-induced decline in haemoglobin oxygen saturation.

Discussion

Both the degree of anaemia and the degree of haemolysis, as indicated by a haemolytic component derived from four markers, were found to be independent predictors of lower steady state oxygen saturation and of oxygen-induced decline in saturation in children and adolescents with sickle cell disease. Increasing age was an additional independent predictor of lower steady state oxygen saturation. Lower values for FEV1, FVC, FEV1/FVC ratio and TLC were not independent predictors of lower steady state oxygen saturation or exercise-induced reduction in saturation. Neither lower steady state oxygen saturation nor desaturation during exercise were predictors of stroke, acute chest syndrome or severe pain history.

Lower oxygen saturation in the setting of sickle cell disease at steady state might be due to several factors including intrinsic lung disease leading to reduced gas exchange and alterations

in the pulmonary vasculature or airways leading to ventilation-perfusion mismatching. Further, altered affinity of haemoglobin for oxygen and increased levels of methaemoglobin or carboxyhaemoglobin may be other contributing factors (Hurford & Kratz, 2004). Several factors could contribute to decreased oxygen affinity, including the intrinsic reduced affinity of haemoglobin S (Seakins *et al*, 1973; Ueda *et al*, 1979), an increase in red blood cell 2,3-biphosphoglycerate related to the degree of anaemia (Milner, 1974) and/or to haemoglobin S polymer formation in the red cells that precedes overt sickling and haemolysis (Sunshine *et al*, 1982). We did not have strong evidence of intrinsic lung disease in the children with sickle cell disease in this study, and the lack of an independent association of lower oxygen saturation with pulmonary function test abnormalities or histories of asthma, acute chest syndrome, and sleep apnea is also against intrinsic lung disease as a major contributing factor. On the other hand, the independent association of lower haemoglobin with lower oxygen saturation might plausibly be related to increased levels of red cell 2, 3-biphosphoglycerate and the independent association with haemolysis to increased haemoglobin S polymer formation. In addition, our finding of an association of greater haemolysis with lower oxygen saturation in all of the statistical analyses that we performed was consistent with the possibility that haemolysis induces changes in the pulmonary vasculature and airways that limit the acquisition of oxygen by haemoglobin in sickle cell disease, thus supporting ventilation-perfusion mismatching as a consequence of haemolysis-induced pulmonary vasculopathy (Kato *et al*, 2007a).

This study is limited by the fact that arterial blood gases were not performed to confirm pO_2 and oxygen saturation, and some evidence suggests that pulse oximetry is not reliable in sickle cell disease (Blaisdell *et al*, 2000). However, a number of other studies have indicated that pulse oximetry is reliable for diagnosing hypoxia during steady state conditions and with acute complications in sickle cell disease patients (Rackoff *et al*, 1993; Kress *et al*, 1999; Ortiz *et al*, 1999). Another limitation of our study is that it is cross-sectional rather than longitudinal. Nevertheless, the findings presented here confirm, in one prospective study, the observations of a number of other investigators. In particular, other investigations have also shown relationships of lower oxygen saturation in sickle cell disease patients with severe anaemia, more severe sickling phenotype (haemoglobins SS and Sβ^0 thalassemia) (Rackoff *et al*, 1993; Homi *et al*, 1997) and/or with haemolysis (Setty *et al*, 2003; Quinn & Ahmad, 2005; Kato *et al*, 2006; Pashankar *et al*, 2008; Quinn & Sargent, 2008; Liem *et al*, 2009). For example, the Dallas Newborn Cohort Study of 585 patients showed independent associations of both the haemolytic marker, reticulocyte count, and low haemoglobin concentration with lower oxygen saturation, with the haemolytic marker explaining more of the oxygen saturation than the haemoglobin concentration (Quinn & Ahmad, 2005); our study was able to duplicate these observations. Our findings point to a potential interrelationship between haemolysis and hypoxia in predicting certain complications of sickle cell disease. An increase in the haemolytic index of 2 SD was associated with a 1·4% decrease in the oxygen saturation within our cohort. Increased haemolysis may contribute to the development of hypoxia by inducing changes in the pulmonary vessels and airways that lead to ventilation-perfusion mismatching. In turn, hypoxia may contribute to the development of haemolytic complications, such as pulmonary hypertension.

Patients in the present study with the lower steady state oxygen saturation or ≥3% reduction in oxygen saturation with exercise had similar statistically significant clinical and laboratory correlates (i.e. markers of haemolysis, lower haemoglobin concentration and higher tricuspid regurgitation velocity). Surrogate markers for diagnosing pulmonary hypertension and predicting its severity in sickle cell disease adult patients are being developed (Machado *et al*, 2006). However, there are no models for predicting the early development of pulmonary hypertension in children (Kato *et al*, 2007b). Inferring from the results of our study, lower steady state oxygen saturation and an acute decline of 3% in oxygen saturation from baseline during the six-minute walk test may serve as early markers for future development of

pulmonary hypertension in children. Therefore, future studies are warranted to longitudinally follow children and adolescents with sickle cell disease.

In conclusion, our findings provide evidence that low haemoglobin oxygen saturation should be considered as a potential feature of the haemolytic vasculopathy sub-pheno-type of sickle cell disease. Haemolysis-related pulmonary vascular dysfunction may underlie both oxygen desaturation through ventilation/perfusion mismatch and shunting as well as pulmonary hypertension.

Acknowledgments

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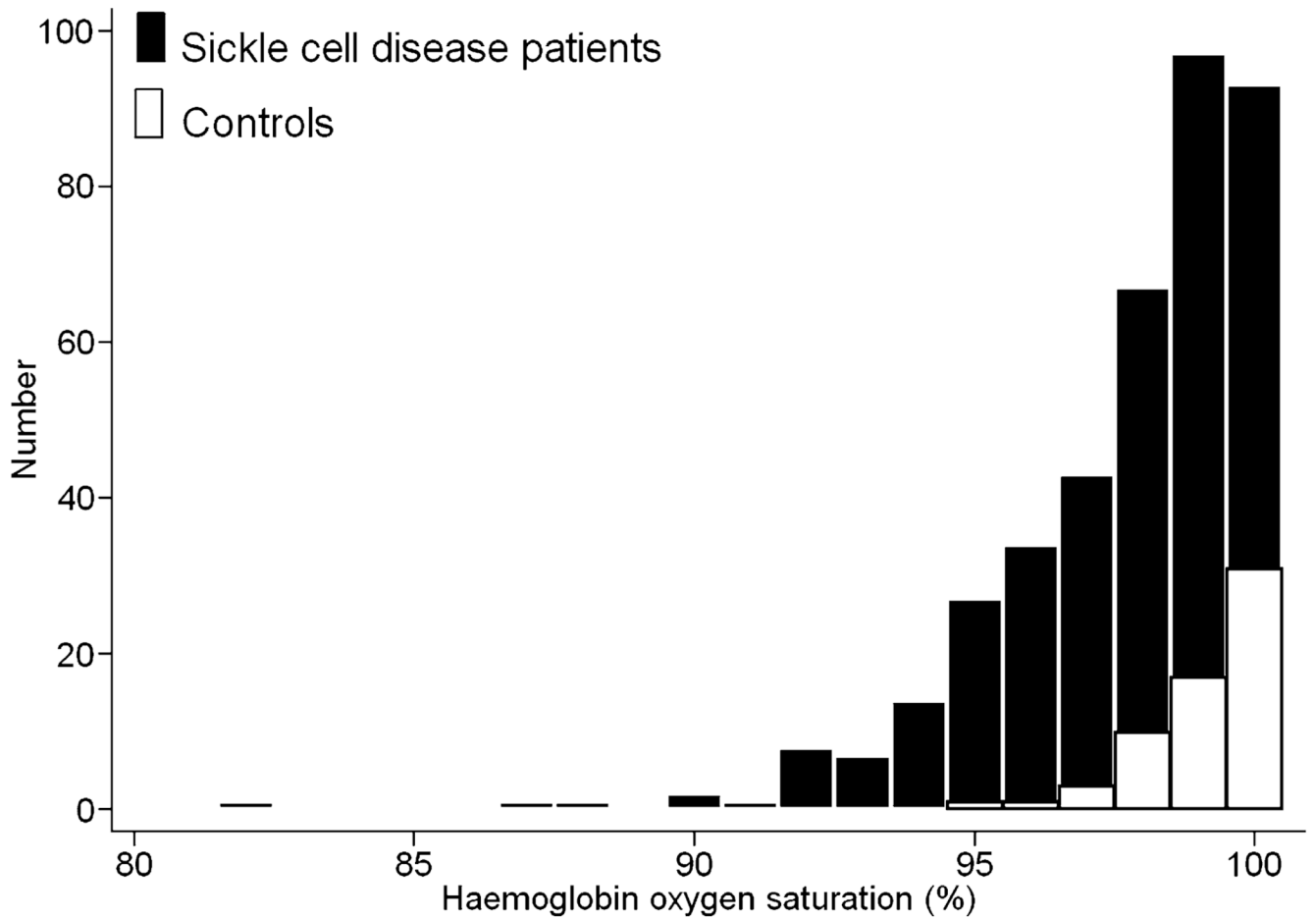
Fig 1.
Steady-state haemoglobin oxygen saturation in patients with sickle cell disease and controls.
The steady state distribution of haemoglobin oxygen saturation in 391 children with sickle cell
disease and 63 controls is depicted. Two hundred and two (52%) of the patients with sickle
cell disease compared to 15 (24%) of the controls had haemoglobin oxygen saturation <99%
while 9% of patients *versus* no controls had saturation <95% ($P = 0·008$).

Table I

Clinical data by haemoglobin oxygen saturation group. Results in median and interquartile range unless otherwise indicated

	NO₂ sat. > 98%	NO₂ sat. 95–98%	N	O₂ sat. < 95%	P
Potential predictors of O₂ saturation					
Age (years)	189 12 (7–16)	168 13 (8–16)	34	12 (8–16)	0·7†
Female sex*	189 104 (55%)	168 69 (41%)	34	17 (50%)	0·030‡
Severe sickling phenotype (Hb SS, Sβ⁰ thal or SD^{L-A})*	185 119 (64%)	167 137 (82%)	34	34 (100%)	<0·0001‡
Regular transfusion programme*	183 25 (14%)	162 24 (15%)	32	4 (13%)	0·9†
Weight for age Z score*	175 0·2 (−0·5–1·0)	161 0 (−0·8–0·8)	34	−0·5 (−1·1–0·1)	0·002†
Hydroxycarbamide therapy*	185 68 (37%)	163 70 (43%)	32	6 (19%)	0·033‡
History of asthma*	186 43 (23%)	163 45 (28%)	31	9 (29%)	0·6‡
History of obstructive sleep apnea*	186 19 (10%)	163 15 (9%)	32	6 (19%)	0·3‡
Haemoglobin (g/l)	184 98 (88–111)	159 88 (79–99)	33	78 (69–85)	<0·0001†
Lactate dehydrogenase (u/l)	168 334 (250–461)	150 411 (300–549)	32	552 (458–694)	<0·0001†
Reticulocytes (× 10⁹/l)	171 173 (127–255)	159 244 (168–351)	33	298 (248–435)	<0·0001†
Aspartate amino-transferase (u/l)	183 35 (27–50)	161 42 (31–53)	34	53 (46–66)	<0·0001†
Total bilirubin (μmol/l)	184 29 (19–46)	160 43 (29–68)	34	56 (39–82)	<0·0001†
Haemolytic component	160 −0·6 (−1·6–0·8)	145 0·4 (−0·7–1·3)	31	1·3 (0·5–2·4)	<0·0001†
FEV1/FVC	64 84 (81–89)	55 86 (80–89)	18	84 (79–87)	0·5†
TLC	47 104 (94–114)	36 103 (89–111)	14	88 (84–92)	0·006†
DLCO	47 94 (85–105)	38 102 (88–113)	13	93 (91–98)	0·1†
Potential outcomes associated with level of O₂ saturation					
TRV (m/s)	167 2·3 (2·1–2·4)	149 2·4 (2·2–2·5)	30	2·4 (2·3–2·5)	0·003†
TRV ≥ 2·6*	167 10 (6%)	149 23 (15%)	30	5 (17%)	0·016‡
Mitral valve E/Etdi	173 6·3 (5·5–7·5)	157 6·4 (5·6–7·3)	33	6·6 (5·7–8·2)	0·3†
Mitral valve E/Etdi ≥ 8·9*	173 16 (9%)	157 9 (6%)	33	4 (12%)	0·3‡
Six-minute walk distance (m)	146 451 (402–508)	141 433 (388–501)	28	478 (398–520)	0·4§
Decline in oxygen saturation of ≥3% during walk	146 7 (5%)	140 13 (9%)	27	7 (26%)	0·001
History of acute chest syndrome*	186 51 (27%)	163 53 (32%)	32	8 (25%)	0·5‡
History of ≥2 severe pain episodes past year*	189 55 (29%)	168 55 (33%)	34	7 (21%)	0·3‡
History >20 units of blood transfused*	185 25 (14%)	160 19 (12%)	32	6 (19%)	0·6‡
History of stroke*	185 18 (10%)	163 17 (10%)	31	6 (19%)	0·3‡
History of priapism*	81 18 (22%)	92 18 (20%)	15	2 (13%)	0·7‡

FEV, forced expiratory volume in 1 s; FVC, forced vital capacity; TLC, total lung capacity; DLCO, Diffusion capacity for carbon monoxide; TRV, tricuspid regurgitation velocity; E/Etdi, E wave to tissue Doppler E wave.

*
Number (%).

†
From ANOVA.

‡
From Chi-square test.

§
Adjusted for height.

Table II

Independent predictors of steady state haemoglobin oxygen saturation among 391 sickle cell disease patients by multiple linear regression analysis[*].

	Beta	95% Confidence interval	*P* value
Haemolytic component (1 SD increase)	−0·71	−0·97 to −0·45	<0·0001
Haemoglobin (10 g/l decrease)	−0·25	−0·11 to −0·40	0·001
Age (1 year increase)	−0·04	−0·08 to −0·001	0·043

FEV, forced expiratory volume in 1 s; FVC, forced vital capacity; TLC, total lung capacity; DLCO, diffusion capacity for carbon monoxide; SD, standard deviation.

[*] r-square = 0·25 for model. The following variables were entered into the model initially: sex, age, hydroxycarbamide therapy, history of asthma, history of sleep apnea, weight for age z-score, haemoglobin, haemolytic component, TLC, FEV1/FVC and DLCO.

Table III

Clinical and laboratory results by six-minute walk-induced decline in oxygen saturation groups among participants with sickle cell disease (results in median and interquartile range unless otherwise indicated)

	N	O$_2$ saturation reduction < 3	N	O$_2$ saturation reduction ≥ 3	P value
Potential predictors of exercise-induced decline in oxygen saturation					
Age (year) *	296	13 (9–17)	27	11 (9–14)	0·030[†]
Female sex *	296	145 (49%)	27	9 (33%)	0·12[‡]
Severe sickling phenotype (Hb SS, Sβ[0] thal or SD^{LA}) *	292	214 (73%)	27	25 (93%)	0·027[‡]
Weight for age Z score *	271	0·1 (−0·7–0·8)	26	−0·4 (−1·1–0·2)	0·003[†]
Regular transfusion programme *	287	38 (13%)	27	6 (22%)	0·2[‡]
Hydroxycarbamide therapy *	291	123 (42%)	27	8 (27%)	0·2[‡]
History of asthma *	291	72 (25%)	27	9 (33%)	0·3[‡]
History of obstructive sleep apnea *	292	28 (10%)	27	5 (19%)	0·15[‡]
History of >20 units of blood transfused *	289	42 (15%)	27	1 (4%)	0·12[‡]
Baseline oxygen saturation (%)	296	99 (97–100)	27	97 (96–99)	0·010[‡]
Haemoglobin (g/l)	285	94 (83–108)	26	75 (69–83)	<0·0001[†]
Haemolytic component	261	−0·1 (−1·2–0·9)	24	1·1 (0·3–2·4)	<0·0001[†]
FEV1/FVC	112	84 (80–89)	17	85 (79–87)	0·4[†]
TLC	80	103 (91–112)	12	92 (81–107)	0·2[†]
DLCO	79	97 (86–109)	14	103 (92–113)	0·4[†]
Potential outcomes associated with exercise-induced decline in oxygen saturation					
TRV (m/s)	257	2·3 (2·1–2·5)	24	2·4 (2·2–2·6)	0·022[†]
TRV ≥ 2·6 *	257	26 (10·1%)	24	7 (29·2%)	0·006[‡]
Mitral valve E/Etdi	267	6·3 (5·5–7·4)	25	6·9 (6·0–7·4)	0·1[†]
Mitral valve E/Etdi ≥8·9 *	267	21 (7·9%)	25	3 (12·0%)	0·5[‡]
Six-minute walk distance (m) *	296	443 (402–504)	27	431 (357–515)	0·8[§]
History of acute chest syndrome *	292	85 (29%)	27	9 (33%)	0·6[‡]
History of stroke *	290	30 (10%)	27	2 (7%)	0·6[‡]
History of priapism *	145	26 (18%)	18	6 (33%)	0·12[‡]

FEV, forced expiratory volume in 1 s; FVC, forced vital capacity; TLC, total lung capacity; DLCO, diffusion capacity for carbon monoxide; TRV, tricuspid regurgitation velocity; E/Etdi, E wave to tissue Doppler E wave.

*
Number (%).

[†]
From Student's t-test.

[‡]
Chi-square test.

[§]
Adjusted for height.

Table IV

Independent predictors of decline in haemoglobin O_2 saturation of three percentage points or more during the six-minute walk among 323 patients by logistic regression[*].

	Odds ratio	95% CI	*P* value
Haemolytic index (increase by 1 SD)	2·5	1·4–4·7	0·003
Haemoglobin (decrease by 10 g/l)	3·3	1·8–6·3	0·0003

FEV, forced expiratory volume in 1 s; FVC, forced vital capacity; TLC, total lung capacity; DLCO, diffusion capacity for carbon monoxide; SD, standard deviation.

[*] Variables entered into model: baseline O_2 saturation, age, height, sex, history of acute chest syndrome, history of asthma, haemoglobin, haemolytic index, TLC, FEV1/FVC, DLCO. Seven cases with residuals out of range were excluded. Area under the curve for the Receiver-operated characteristic = 0·94. *P* for goodness of fit = 0·99.