Development of pulmonary function abnormalities in sickle cell disease

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Development of pulmonary function abnormalities in sickle cell disease

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For the degree of PhD

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**Author Statement**

I state that the work presented in this thesis is original and my own work. During my research degree I was supervised by Professor Anne Greenough and Dr Gerrard F Rafferty. Dr Catherine Wedderburn, Dr Na’eem Ahmed, Dr Polly Robinson and Miss Emily McGhee assisted with recruitment and testing of the children with SCD at various stages. Professor David Hansell and Dr Sujal Desai scored the parenchymal abnormalities on the CT scans presented in chapter four, and echocardiography was performed by Dr Sitali Mushami.
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I owe a huge debt of gratitude to the patients and healthy volunteers who gave up their time to participate in this work, and to the staff and students of Burntwood School who took time out of their school day on more than one occasion to participate in the longitudinal study.

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Abstract

Sickle cell disease (SCD) is one of the commonest inherited disorders worldwide. Acute chest syndrome (ACS) is the commonest cause of death in young adults and pulmonary dysfunction is a major contributor to morbidity in aging adults with SCD, but the aetiology is poorly understood. The first four studies presented in this thesis test the hypothesis that pulmonary vascular abnormalities are involved in the pathogenesis of SCD-related lung disease.

Significant associations were found between small vessel pulmonary vascular dimensions, lung function abnormalities and echocardiographic estimates of ventricular function and cardiac output in adults with SCD; the decline in lung function observed in a subset of patients with longitudinal measurements correlated with changes in vascular dimension. Increased pulmonary capillary blood volume was shown to be related to the airways obstruction seen in SCD children. Respiratory system resistance and pulmonary capillary blood volume were significantly correlated in the SCD children, but not in the controls, suggesting the raised respiratory system resistance in the SCD children was, at least partly, explained by their increased pulmonary capillary blood volume. Additionally, fluid loading by means of blood transfusion in SCD children was found to acutely increase pulmonary capillary blood volume resulting in an increase in respiratory system resistance and worsening lung function. Furthermore, alveolar nitric oxide production was elevated in SCD children compared to controls and in the SCD children was correlated with pulmonary blood flow, but airway nitric oxide flux was
similar to that of the controls and did not correlate with biomarkers of airways obstruction.

Lung function appears to deteriorate over time in children with SCD, but prospective longitudinal data with appropriate control groups were lacking. The study presented in chapter five tested the hypothesis that lung function in SCD children, assessed longitudinally, would decline relative to controls and was related to ACS, and that restrictive abnormalities would increase in prevalence. In SCD children, but not controls, lung function declined significantly, and was significantly associated with ACS. Restrictive abnormalities were significantly more common at follow up.
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List of abbreviations

$\Theta_{CO}$ rate of uptake of carbon monoxide by whole blood and combination with Hb

A/B ratio ratio of segmental pulmonary artery and bronchus diameter

ACS acute chest syndrome

ATS American Thoracic Society

$C_{A,NO}$ alveolar concentration of nitric oxide

CO cardiac output

CD36 cluster of differentiation 36

CO carbon monoxide

$CO_2$ carbon dioxide

$CO_{alv(t=0)}$ concentration of carbon monoxide prior to transfer across the alveolar-capillary membrane

$CO_{alv(t=10)}$ expired concentration of carbon monoxide after ten seconds breath-holding

CSA<5mm% Total cross sectional area of all pulmonary vessels less than 5mm in diameter as a percentage of CT slice area

CTPH chronic thromboembolic pulmonary hypertension

$D_L CO$ total lung gas transfer for carbon monoxide

$D_L COc$ total lung gas transfer for carbon monoxide corrected for haemoglobin concentration

$D_L NO$ total lung gas transfer for nitric oxide

$D_m CO$ membrane diffusion coefficient for carbon monoxide

$D_m NO$ membrane diffusion coefficient for nitric oxide

E early diastolic LV inflow velocity

e’ lateral mitral annulus velocity

$E/e’$ ratio of early diastolic LV inflow velocity to lateral mitral annulus velocity

ERS European Respiratory Society

ERV Expiratory reserve volume
**FEF<sub>25-75</sub>** forced expiratory flow between 25 and 75% of VC exhaled

**fe<sub>NO,50l/s</sub>** fractional exhaled nitric oxide measured at an exhalation rate of 50ml/s

**FEV<sub>1</sub>** forced expiratory volume in one second

**FVC** forced vital capacity

**FEV<sub>1</sub>:FVC** ratio of forced expiratory volume in one second to forced vital capacity

**FRC** Functional residual capacity

**Hb** haemoglobin

**HbA** adult haemoglobin

**HbC** haemoglobin C

**HbF** fetal haemoglobin

**HRCT** high resolution computed tomography

**HbS** sickle haemoglobin

**HbS β-thal** sickle cell β-thalassemia

**HbSC** heterozygous HbS-HbC disease

**HbSS** homozygous sickle cell disease

**He** helium

**ICAM-1** intercellular adhesion molecule-1

**IL-1** interleukin-1

**IOS** impulse oscillometry

**J'aw,NO** maximal airway flux of nitric oxide

**KCO** gas transfer per unit lung volume

**kCO** rate constant for carbon monoxide uptake

**KCOc** gas transfer per unit lung volume corrected for haemoglobin concentration

**LDH** lactate dehydrogenase

**LLN** lower limit of normal

**LMM** linear mixed model

**N<sub>2</sub>O** nitrous oxide

**NF-κB** nuclear factor κB
NO  | nitric oxide  
O₂  | oxygen  
PASP | pulmonary artery systolic pressure  
Pb  | barometric pressure  
PH₂O | partial pressure of water vapour  
ppb | parts per billion  
ppm | parts per million  
QIₚᵤₐₙₜ | pulmonary blood flow index  
Qₚᵤₜₐₜ | pulmonary blood flow  
Rrs(0) | mean respiratory system resistance  
Rrs(1) | frequency dependence of resistance from 5-20Hz  
Rrs₅ | respiratory system resistance at an oscillation frequency of 5Hz  
RV | residual volume  
RV:TLC | ratio of residual volume to total lung capacity  
RVDV | right ventricular diastolic volume  
SCD | sickle cell disease  
SCLD | sickle chronic lung disease  
SF₆ | sulphur hexafluoride  
SpO₂ | oxygen saturation by pulse oximetry  
TAPSE | tricuspid annular plane systolic excursion  
TLC | total lung capacity  
TLV<sub>CT</sub> | total lung volume derived from CT  
TNF-α | tumour necrosis factor alpha  
TRV | tricuspid regurgitant jet velocity  
ULN | upper limit of normal  
V<sub>A</sub> | alveolar volume  
V'<sub>A,NO</sub> | alveolar nitric oxide production  
VC | vital capacity  
V<sub>c</sub> | Pulmonary capillary blood volume  
VCAM-1 | vascular cell adhesion molecule-1  
جالبة | rate of exhaled nitric oxide in nl/s
1 INTRODUCTION

1.1 Sickle cell disease

Sickle cell disease (SCD) is an inherited haematological disorder that is associated with multisystem organ damage, and is one of the commonest monogenetic diseases worldwide [1]. SCD was first described in 1910 by James Herrick when he observed “peculiar elongated and sickle-shaped red blood corpuscles” in blood films from an African-American patient presenting with severe anaemia [2]. Since that time, our understanding of this condition has progressed rapidly. In 1949 Linus Pauling et al described SCD as “the first molecular disease” when they detected distinct electrophoretic anomalies in sickle haemoglobin [3], and this work formed the basis of the subsequent detailed characterisation of the fundamental genetic and biophysical basis of the condition. With improved general healthcare the majority of patients with SCD in developed countries can expect to survive to adulthood, but despite the progress made, specific treatment options available for clinical management of the disease are limited. This is, at least in part, due to the highly varied and complex clinical presentation and underlying pathophysiology seen in individual patients [4]. The work presented in this thesis aims to contribute to the understanding of the pathophysiology of the pulmonary complications in adults and children with SCD and hence aid in the determination of possible preventative interventions.
1.2 Incidence of SCD

SCD occurs most frequently in people of African origin, although it is also present in populations originating in India, the Middle-East, Latin America, and Southern Europe [5]. The heterozygous carrier state is thought to confer a degree of protection against malaria and the distribution of SCD is to a large extent coincident with that of malaria [5]. Approximately three hundred thousand infants are born with SCD per year worldwide and the large-scale migration from Africa and Asia in the nineteenth and twentieth centuries has resulted in a substantial incidence of SCD in Western Europe and the United States. In the United Kingdom, SCD is now as common as cystic fibrosis with a prevalence of approximately 1:2000 affected births, mostly concentrated in London and represents a substantial healthcare burden (figure 1.1) [6].
Figure 1-1 Geographical distribution of sickle cell disease in England: annual rate (per 1000 births) by county district.

Adapted from Hickman et al 2008
1.3 The pathophysiology of SCD

Sickle cell disease refers to the clinical syndrome caused by one of a number of single-gene mutations and the resultant production of an abnormal haemoglobin variant, the commonest of which is haemoglobin-S (HbS). Individuals who are homozygous for HbS are said to have sickle cell anaemia (HbSS) and constitute the majority of the SCD population, comprising approximately seventy percent of cases in populations of African heritage. The majority of the remainder have haemoglobin SC disease (HbSC) resulting from the presence of the HbS and HbC alleles [7]. HbS/β-thalassemia, which occurs when HbS is inherited with one of a number of β-thalassemia alleles [8], is the third major group. The data presented in this thesis relate to HbSS disease.

HbS results from a single point mutation in the gene which codes for β-globin whereby the seventeenth nucleotide, thymine, is substituted with adenine resulting in the replacement of glutamic acid with valine in the sixth position of the β-globin chain [9]. This results in a hydrophobic structural motif in the deoxygenated HbS tetramer which allows binding between the β1 and β2 chains of different haemoglobin molecules and thereby provides a polymer nucleus, with subsequent polymerization of HbS throughout the erythrocyte. The resulting disturbance of cellular architecture reduces the flexibility of the erythrocyte and promotes water loss, resulting in mechanical and oxidative stress [10]. The key determinants of the extent and rapidity of this polymerization process are the intracellular HbS concentration, the degree and duration of haemoglobin
deoxygenation, and the concentration of fetal haemoglobin (HbF) which mitigates HbS polymerization by reducing the average intracellular HbS concentration [11]. Hereditary persistence of high HbF concentrations can ameliorate disease severity and induction of elevated HbF levels are a mainstay of SCD pharmacotherapy. HbS polymers cause damage to erythrocytes and the erythrocyte membrane [12], resulting in abnormal rheological and hemodynamic properties characterized by increased cell density, bulk viscosity, and peripheral resistance [13]. There is considerable variability which is thought to be attributable to between-patient and between-erythrocyte variations in intracellular HbF distribution [14, 15]. In some erythrocytes, ion homeostasis is disrupted; loss of water and potassium ions results in an increased cell density. That process is mediated by the activation of channels such as the K⁺:CL⁻ co-transporter and the potassium intermediate/small conductance calcium-activated channel (Gardos channel), in response to acidification, deoxygenation, influx of calcium ions, and erythrocyte sickling [16-18]. Damage to the erythrocyte membrane also results in the release of lipid-encapsulated micro-particles [19, 20]. HbS polymerization, and the injured sickle erythrocytes thereby produced, is the central pathophysiological event in SCD and manifests in two important disease processes: vaso-occlusion (and subsequent reperfusion injury), and chronic haemolytic anaemia.
1.4 Vaso-occlusion in SCD

The conformal changes that are caused by intracellular HbS polymerization result in erythrocytes that are less deformable than normal cells and this may result in the trapping of sickled cells in the microcirculation and consequent vascular obstruction. This process is central to the acute painful crisis which is one of the commonest clinical manifestations of SCD. Painful crises are often triggered by inflammatory processes and are the result of complex interactions between erythrocytes, leucocytes, plasma proteins, endogenous vaso-active substances and the vascular endothelium [21]. They are mediated by adhesion molecules including $\alpha_4\beta_1$ integrin, CD36, the Lutheran blood group antigen, and phosphatidylserine [22-26]. Both sickled and un-sickled erythrocytes also bind to leucocytes or platelets to produce cellular aggregates which substantially increase the likelihood of obstruction of the vascular lumen [21, 27, 28]; the frequency of vaso-occlusive events has been shown to increase as the leukocyte count increases [29, 30].

Leukocytes play a role in SCD vaso-occlusion via interaction with the vascular endothelium and by aggregation with each other and with different cell types [21]. The formation of cellular aggregates by leucocytes, together with increased adherence to vascular endothelium is mediated by various adhesion molecules and high levels of the leucocyte adhesion molecules $\alpha M\beta 2$ integrin and L-selectin have been linked to more severe clinical expression of SCD [31]. These interactions are increased by inflammation and initiate vascular obstruction in the post capillary venules [21, 32-34]. An indirect role for leukocytes in vaso-occlusive episodes in
SCD via activation of the vascular endothelium has also been demonstrated. Monocytes activate vascular endothelium via the release of inflammatory cytokines including tumour necrosis factor alpha (TNF-α) and interleukin-1β [35]. Activated endothelium up-regulates the expression of ligands for leukocyte and erythrocyte adhesion molecules including intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) [36, 37]. By these mechanisms the adhesion of circulating blood cells is enhanced by monocyte action and the propensity for vaso-occlusion increased. Vascular occlusion leads to ischaemia and subsequent reperfusion injury when the circulation is restored. Repeated episodes of ischaemia and reperfusion result in increased oxidative and inflammatory stress, leading to vascular oxidase activation and further endothelial cell-adhesion molecule expression and a corresponding increase in inflammatory cytokines which may result in leucocytosis [21, 33, 38, 39]. Bone marrow infarction and the formation of fat emboli have also been linked to vaso-occlusion [40].
1.5 Haemolysis in SCD

Abnormal sickle erythrocytes have a reduced lifespan and consequently, individuals with SCD suffer from haemolytic anaemia. The degree of anaemia varies widely between SCD genotypes; it is most severe in HbSS disease and mildest in HbSC disease and HbS-β⁺ thalassemia. Considerable variation is also seen within genotypes. In HbSS disease, red cell survival varies between two and twenty-one days and this results in a similarly wide range in clinical markers of haemolysis, such as lactate dehydrogenase (LDH), bilirubin level, reticulocyte count and haemoglobin concentration [41]. Most haemolytic activity is extravascular and results from phagocytosis by reticulo-endothelial cells which are able to recognise damaged erythrocytes [42, 43]. Substantial intra-vascular haemolysis, however, can also occur and may constitute up to thirty percent of the total erythrocyte turnover [44, 45]. Sustained extra-vascular haemolysis may result in saturation of Hb-binding proteins, predominantly haptoglobin, with a consequent increase in circulating cell-free haemoglobin. In addition to chronic anaemia, high haemolytic rates and chronically low haemoglobin concentration are associated with a number of distinct clinical complications, including cutaneous leg ulcers, cholelithiasis, priapism and pulmonary hypertension [46-48]. Furthermore, haemolysis has been implicated in the development of progressive vasculopathy, which typically features endothelial dysfunction, hypertension and vascular remodelling characterised by smooth muscle and intimal proliferation [48-51]. Increased severity of haemolytic anaemia has also been associated with the development of echo-cardiographically defined pulmonary hypertension in adults [48, 52, 53] and
children with SCD [54-56]. Kato et al., proposed that individuals with SCD with high haemolytic rates and correspondingly low stable haemoglobin concentrations constitute a distinct sub-phenotype with an increased likelihood of developing vasculopathy, in contrast to those patients with higher steady-state haemoglobin concentration and increased blood viscosity who are predisposed to vaso-occlusive pathology such as acute pain crisis and acute chest syndrome [46]. The increase in cell-free haemoglobin resulting from intravascular haemolysis has important consequences for vascular homeostasis. Cell-free circulating haemoglobin generates reactive oxygen species including the hydroxyl and superoxide radical [57] – a potent scavenger of nitric oxide [45, 58]. Endothelium-derived nitric oxide is a key regulator of basal vasomotor tone, in addition to inhibiting platelet and haemostatic activation and transcriptional expression of nuclear factor κB (NF-κB)-dependent adhesion molecules including ICAM-1 and VCAM-1, as well as the selectins [59-62]. By means of scavenging, haemolysis results in the rapid binding of NO and the formation of methaemoglobin, thereby inhibiting endothelial nitric oxide signalling and resulting in endothelial cell dysfunction and nitric oxide resistance [45, 47, 63]. Furthermore, the release of erythrocyte arginase-1 reduces plasma arginine levels and thereby decreases the substrate required for synthesis of nitric oxide, further reducing nitric oxide bioavailability [64]. Furthermore, chronically reduced NO bioavailability may play a role in the hyper-coagulable state found in individuals with SCD, as NO has been shown to have a role in the suppression of platelet aggregation and pro-coagulant proteins [45, 65-67]. The haemolytic rate has been shown to correlate with platelet activation levels and plasma pro-coagulant factors [68, 69].
1.6 Pulmonary complications of SCD

The respiratory system is particularly vulnerable to the pathophysiological processes active in SCD. Pulmonary pathology is commonly found in cases of sudden death in SCD patients. One study reported that over 70% of patients who died suddenly during an admission for SCD exhibited pulmonary pathology on post-mortem. In the patients with pulmonary pathology, evidence of pulmonary thromboembolism was found in 47%, pulmonary oedema in 41%, fat emboli in 33%, right ventricular hypertrophy in 33%, grades I – IV pulmonary arterial hypertension in 33% and microvascular thrombi in 29% of subjects [70]. Pulmonary manifestations of SCD, in particular the acute chest syndrome (ACS), and sickle chronic lung disease (SCLD) have high mortality rates.
1.6.1 Sickle chronic lung disease

Adults with sickle cell disease can suffer from parenchymal lung disease, pulmonary vascular disease, or both. This spectrum of abnormalities is commonly referred to as ‘sickle chronic lung disease’ (SCLD). In some cases, these processes may progress to restrictive lung disease, and/or pulmonary hypertension. There is no curative treatment for SCLD, but ACS is the most significant risk factor.

Restrictive lung disease/pulmonary fibrosis

An early case series of chest radiographs from twenty-six patients with SCD of mixed genotype described scattered areas of lung fibrosis [71] in conjunction with segmental infarction in more than one-third of cases; these abnormalities were more pronounced in patients who had suffered repeat episodes of ACS and were associated with an increased risk of early death [71]. More recently, high-resolution computed tomography (HRCT) has been used to evaluate parenchymal abnormalities in adults with SCD. One study showed that approximately forty percent of patients had evidence of interstitial disease on HRCT, however, no significant relationships between pulmonary function test results and HRCT abnormalities were observed [72]. The interval between pulmonary function testing and HRCT examination, however, was large, ranging from one month to more than one year. A more recent study from our research group in which the lung function assessments and HRCT were conducted contemporaneously, demonstrated that the majority of a cohort of adult patients with SCD had
pulmonary vascular or parenchymal abnormalities on high-resolution CT (HRCT) [73] and the HRCT findings were significantly correlated with pulmonary function testing results. Of particular note, there were correlations between reductions in forced vital capacity (FVC) and forced expiratory volume in one second (FEV₁) and a qualitative scoring of prominence of the central vessels on HRCT. Prominent central vessels were found on HRCT in eight of the nine patients with restrictive abnormalities. The authors speculated that the prominent central vessels might reflect changes related to pulmonary hypertension suggesting a possible vascular origin for pulmonary dysfunction.

**Pulmonary hypertension**

In recent years, pulmonary arterial hypertension has emerged as a cause of morbidity and mortality in adults with SCD [48, 74, 75]. Chronic haemolysis may lead to reduced nitric oxide bioavailability and it has, therefore, been hypothesised that depletion of this important vasodilator in the pulmonary vascular bed leads to an increase in pulmonary vascular resistance and a consequent increase in pulmonary artery pressure, with deleterious long term effects [48]. Subsequent work has, however, called this hypothesis into question. The initial large screening study by Gladwin et al [48] used an elevated tricuspid regurgitant jet velocity (TRV) derived from echocardiography as a surrogate marker for an abnormally high pulmonary artery pressure (a TRV of ≥ 2.5 m/s was deemed to be abnormal) and observed that more than thirty percent of patients with SCD in their unselected cohort may have had pulmonary arterial hypertension. They also found that this
was linked to an increased likelihood of premature death [48]. This study was followed by others which also found a comparable prevalence of elevated TRV [52, 53, 76]. More recent studies have used right-heart catheterisation to directly measure pulmonary arterial pressures and pulmonary vascular resistance in adults with SCD and elevated TRV and have reported a much lower prevalence of pulmonary hypertension [75, 77]. In a large study (n = 398), Parent et al reported that only six percent of SCD patients had pulmonary hypertension and that in over fifty percent of patients, this was pulmonary venous, rather than pulmonary arterial hypertension. It is at present not known if the increased mortality observed in SCD patients with elevated TRV in the large screening studies [48, 52, 53, 76] is due solely to the subset of patients with pulmonary hypertension.
1.6.2 The Acute Chest Syndrome

ACS, defined as radiological evidence of new pulmonary infiltrates consistent with consolidation together with fever, chest pain, tachypnoea, cough, or wheezing [40, 78, 79], is an acute lung injury syndrome that is common in patients with SCD. ACS is a major source of morbidity and is the second most common cause of hospital admission [40, 80] and the leading cause of premature death [30, 78, 81-83]. The results of one study suggested that the mortality may be related to acute increases in pulmonary artery pressure in patients with underlying pulmonary hypertension and cor-pulmonale, and that ACS acts as a stressor rather than the primary cause of death [84]. The Cooperative Study of Sickle Cell disease reported a twenty-nine percent incidence of ACS over a two-year period in a large cohort of subjects with HbSS disease [82].

ACS often occurs in hospitalised adult patients with SCD, with an incidence of between ten and twenty percent, where it is usually preceded by an acute vaso-occlusive pain crisis [78, 85]. An increased risk of ACS is associated with younger age, higher steady-state haemoglobin concentration, elevated steady-state leucocyte count, lower HBF concentration [86], a history of smoking or smoke exposure [87] and in children, a diagnosis of asthma [83, 86, 88-92]. ACS tends to be more severe in HbSS disease compared to other genotypes [88, 93-95]. The causes of ACS are thought to fall into several main categories which are discussed below.
Infection

In both adults and children, pulmonary infection is implicated in many cases of ACS. In a large cohort of patients in the USA (n = 538), an infectious pathogen was isolated in 54% of admissions for ACS from a total of 671 episodes [78]. These were predominantly atypical bacteria and viruses; community acquired encapsulated bacteria were only found in a minority of cases (< 10% of ACS episodes). A total of twenty-seven different pathogens were identified of which *C. pneumoniae* was the most frequent (isolated in 71 episodes), followed by *M. pneumoniae* (isolated in 51 episodes) and respiratory syncytial virus (isolated in 26 episodes). Parvovirus was identified in ten cases and was associated with severe reticulocytopenia [78]. Compared to patients who were infected with mycoplasma strains, patients with chlamydia infections were significantly more likely to suffer a vaso-occlusive event during their hospital stay, and had higher mean haemoglobin levels [78]. Seasonal influenza infection has also been linked to ACS [96-99]. Furthermore, more than eighty percent of adult patients with SCD report a history of hospital admission due to “pneumonia” and necessitating treatment with intravenous antibiotics [48]. Infection may precipitate ACS due to an enhanced susceptibility to inflammatory provocation. In studies of transgenic mice expressing human HbS, SCD mice exhibited a heightened susceptibility to inflammatory provocation by lipopolysaccharide and bacterial endotoxin, resulting in lung injury at levels of endotoxin that had no adverse effect on wild-type mice [100, 101].
**Fat emboli and ACS**

Fat emboli may play a significant role in the pathogenesis of ACS. Severe vaso-occlusive pain crises, typically involving the proximal skeleton, result in bone marrow infarction and oedema and eventually to necrosis and the release of fatty contents into the bloodstream. These contents then travel to the pulmonary vasculature where they trigger severe lung inflammation, acute pulmonary hypertension and hypoxaemia by means of direct mechanical occlusion and inflammation [102-105]. Fat-laden alveolar macrophages obtained during bronchoscropy are diagnostic of pulmonary fat embolism. In the national ACS study cohort, fat embolism was diagnosed in sixteen percent of ACS cases in adults and children [78] by bronchoscopy. Another study found that patients with ACS in whom lipid-laden macrophages were identified in induced sputum followed a more severe clinical course, with more severe extra-thoracic pain and a greater incidence of neurological symptoms compared to patients without evidence of fat embolus [104].

**Intrapulmonary red cell sequestration/thrombosis**

The adhesion and trapping of sickled erythrocytes is central to the pathophysiology of SCD. The pulmonary vascular bed may be especially vulnerable to this process, as the pulmonary circulation is subject to vaso-constriction under hypoxic conditions. This process is, however, difficult to demonstrate and the prevalence, severity and relative importance of this mechanism is unknown. In a study of 125
patients with SCD during 144 ACS episodes, Mekontso et al found sub-segmental thromboembolism in seventeen percent of cases, with no evidence of peripheral thrombosis and concluded that the pulmonary emboli were due to in-situ cellular occlusion and thrombosis and that these mechanisms may therefore be more important than previously appreciated [84].

Alveolar hypoventilation

Episodes of ACS are frequently temporally related to infarcts of the ribcage [106]. This has led to the suggestion that, due to subsequent restriction of chest wall motion and alterations in breathing pattern due to the pain and discomfort, atelectasis and hypoventilation occur which contribute to the development ACS [107, 108]. A study of breathing patterns adopted by SCD patients with both thoracic and non-thoracic pain found that a pattern of rapid shallow breathing is a feature of patients with thoracic bone pain [109]. Radiological studies have also highlighted an association between rib-infarction and radiographic changes of ACS [106, 108, 110]. In SCD children admitted to hospital with acute back and/or chest pain, incentive spirometry has been used to improve ventilation and to significantly reduce the incidence of acute pulmonary complications during the period of admission [111]. The use of opiates for analgesia during painful crisis has also been linked to hypoventilation and development of ACS [112].
Asthma/wheezing

Asthma has been linked to an increased risk of ACS in children with SCD. In a retrospective review of 139 children with SCD, Boyd et al found that children with SCD and asthma had four times as many ACS episodes as non-asthmatics (95% CI, 1.7 - 9.5) together with an increased length of hospitalisation per episode (p = 0.01) [113]. Nordness et al also found, in a retrospective study of ninety-six children with SCD, that those with asthma had a significantly greater frequency of ACS episodes than those without (p = 0.03) [91]. Asthma is also associated with recurrent ACS episodes. In a study of eighty children with SCD, Knight-Madden et al found that asthma (80% v 40%, p = 0.005) and particularly atopic asthma (53% v 12%, p<0.001), were more common in children with SCD who had suffered recurrent episodes of ACS than in those who had suffered a single or no episode [88]. In a subsequent prospective study, Boyd et al found that children with SCD and asthma suffered twice as many ACS episodes as those without asthma (0.39 vs. 0.2 episodes per patient-year, p < 0.001) and that the children with asthma developed their first ACS episode at a significantly younger age (2.4 vs. 4.6 years, p = 0.010) [86]. Those results were consistent with a study by Sylvester et al of 165 children with SCD which found that a greater number of the children who had an ACS (n=83) compared to those who had not were taking anti-asthma medication (p = 0.02). Importantly, the children with a history of ACS had been diagnosed as asthmatic at a median of 3.5 (range 0.5-7) years prior to their first ACS episode, suggesting that asthma may predispose children with SCD to subsequent ACS episodes [83]. Those results were similar to those obtained in a later retrospective analysis of 297
children with ACS which found that SCD children with a diagnosis of asthma had an increased rate of ACS (0.31 events per patient-year vs. 0.16 events per patient-year, p = 0.002) compared to those who did not [93].

Doubt has, however, been cast on the attribution of wheezing symptoms in children with SCD to asthma. Asthma is characterised by variable airflow obstruction and bronchial hyper-reactivity resulting from chronic inflammation [114]. Although a diagnosis is made more reliable by evidence of reversible airflow obstruction on pulmonary function testing, laboratory markers of atopy or inflammation, and/or a family history of atopic or allergic disease, in practice the diagnosis is often made on the basis of the clinical presentation alone [115]. In the majority of the studies of asthma in SCD described above, the diagnosis was made by medical records review [86, 91, 113] or current use of anti-asthma medication [83, 86]. The study by Knight-Madden et al did, however, provide supporting evidence for a diagnosis of asthma by performing skin-prick testing for atopy [88]. Interestingly, whilst a diagnosis of asthma was significantly more common in the SCD children compared to controls (48% vs. 22%, p = 0.002), there was no significant difference in the prevalence of atopy between the SCD and control groups. In asthmatic populations, atopy is in general significantly more common compared to healthy subjects [116, 117].

There is evidence to suggest that wheezing in SCD may be due to disease processes other than asthma. In a study of 187 patients, physician diagnosed asthma was associated with a personal and family history of atopy, but many of the SCD patients had recurrent wheezing and had not been diagnosed with asthma [118].
Furthermore, wheezing symptoms were associated with an increased risk of ACS, irrespective of a diagnosis of asthma [118]. In another study of 262 patients with SCD attending emergency departments, nineteen percent had one or more episodes of physician-documented wheeze and fifty-three percent did not have a diagnosis of asthma [119]. The patients with wheeze had a greater number of attendances for painful crisis and ACS, but those with asthma had a greater number of attendances for painful crisis only. Those data suggest that asthma may be substantially underdiagnosed in children with SCD or that sickle-related wheezing and asthma may be two distinct disease entities with different pathophysiological bases.

Measurement of exhaled nitric oxide (f_{eNO}), which reflects airway NO production and is a marker for eosinophilic airways inflammation, might help to further elucidate whether asthma is increased in SCD children as F_{eNO} is often elevated in patients with asthma [120]. Data on f_{eNO} levels in SCD are, however, sparse. The only published measurements are by Girgis et al, who found that adults with SCD had a significantly lower f_{eNO} than healthy control subjects [121], but the control group was not matched for race which hampers interpretation of the results. Exhaled NO (eNO) has been shown to be elevated in patients with atrial septal defects, a condition which is associated with a hyper-dynamic pulmonary circulation and eNO correlated with increased pulmonary blood flow [122]. Since children and adults with SCD often have a hyper-dynamic circulation due to the raised cardiac output resulting from chronic anaemia [123] any investigation into
f_{eNO} in SCD must consider the potential impact of elevated pulmonary blood flow on f_{eNO} measurements. This was one of the aims of this thesis.

The haemodynamic changes associated with chronic anaemia, characterised by an increased cardiac output and dilation of the pulmonary vasculature, may also provide an alternative explanation for airflow obstruction and wheeze in SCD. Inflation of the leg compartments of pneumatic trousers increases thoracic blood volume and has been shown in healthy volunteers to increase respiratory resistance [124]. In addition, in patients with impaired left ventricular function, acute intravascular volume expansion by means of fluid loading impairs alveolar-capillary membrane function and increases airflow obstruction [125], an effect which has also been observed in healthy subjects [126, 127]. Those results suggest that the airflow obstruction was mediated, at least in part, by dilation of the pulmonary vessels [128]. Children and adults with SCD have an increased pulmonary vascular blood volume which is a response to their chronic anemia resulting in a raised cardiac output and increased vascular recruitment and distension [123, 129, 130], but the possible effect of this on lung function has not been investigated. Therefore, an aim of this thesis was to assess the effects of pulmonary vascular changes related to a hyperdynamic circulation on lung function in adults and children with SCD. This would provide insights into the mechanisms underlying the lung function abnormalities observed in SCD patients and hence aid in the determination of possible preventative interventions.
1.7 Lung function abnormalities in SCD

1.7.1 Cross-sectional studies

In adults

The first measurements of pulmonary function in patients with SCD were made in a cohort comprising six adults with SCD of mixed genotypes and thirty healthy control subjects. Total lung capacity (TLC) and vital capacity (VC) were found to be reduced in the SCD group compared to the controls, suggesting a restrictive defect [129]. The transfer factor for carbon monoxide (DLCO) was elevated in the SCD group when corrected for reduced haemoglobin, due to an increased pulmonary capillary blood volume [131]. Similar results were subsequently described by Miller et al in a study of 25 patients with HbSS disease [132].

In a more recent study by Santoli et al in a cohort of 49 patients with SCD comprising forty-two subjects with HbSS, four with HbSC, and three with HbSβ-thalassemia disease [133], lung function results were classified as normal, restrictive (TLC < 80% predicted), obstructive (ratio of forced expiratory volume in one second (FEV\_1) to forced vital capacity (VC): (FEV\_1: VC) < 80% predicted) or mixed (both TLC and FEV\_1: VC < 80% predicted). A restrictive pattern was the most common finding, observed in twenty (41%) of the patients, whilst eight patients (17%) were obstructive were and ten (20%) had a mixed obstructive/restrictive defect [133]. The prevalence of obstructive and restrictive defects did not differ in those patients with a history of ACS compared to those without, although the ACS
group had a higher DLCO and DLCO per unit lung volume (KCO) when corrected for haemoglobin level. Interestingly, the respiratory system resistance ($R_{rs}$) measured by the forced oscillation technique (FOT) was higher in patients with a history of ACS, leading the authors to speculate that the increase in $R_{rs}$ was related to an increase in pulmonary blood volume [133].

In the largest study of lung function in SCD to date, Klings et al examined lung function test results performed as part of the cooperative study of sickle cell disease from three-hundred and ten adults with HbSS disease [134]. Lung function defects were classified as restrictive, obstructive, mixed obstructive/restrictive, or ‘isolated low DLCO’ according to an American Thoracic Society position statement [135]. Whilst the definition of a restrictive defect was comparable to the criteria used in the study of Santoli et al, in order to be classified as obstructive or mixed the DLCO had to be normal, which precluded the possibility that impaired gas transfer coexisted with airway abnormalities. As a result, the prevalence of obstructive disease may have been underestimated. Nevertheless, that study found a high incidence of restrictive defects (74%). Thirteen patients had an isolated reduction in DLCO, but only four percent were deemed to have an obstructive defect, lower than that found by Santoli et al [133]. Furthermore, no significant difference was observed in those patients with a history of ACS compared to those without, although there was a trend towards a more restrictive pattern ($\text{TLC} = 69.2\%\text{predicted}$ versus $72.8\%\text{predicted}$, $p=0.06$) in the ACS group [134]. On multiple linear regression, a significant association was found between reduced DLCO and thrombocytosis, hepatic dysfunction (as assessed by elevated
alanine aminotransferase), and renal dysfunction (as assessed by elevated blood urea nitrogen and creatinine) leading the authors to conclude that gas exchange dysfunction was likely to be the result of more severe sickle vasculopathy characterised by impaired renal and hepatic function. Sylvester et al classified lung function test results from thirty-three patients with HbSS disease as either normal, restrictive, obstructive, or mixed obstructive/restrictive using diagnostic criteria comparable to those used by Santoli et al. Nine patients (27%) had a restrictive lung function abnormality, five (15%) an obstructive abnormality and four (12%) a mixed restrictive/obstructive abnormality.

All of those studies used fixed percentage cut-offs to define lung function abnormalities. Current American Thoracic Society/European Thoracic Society guidelines recommend that abnormalities are classified according to the lower limit of normal (LNN) based on percentiles [136], as fixed cut-offs do not take into account the fact that the limits of the normal range for all lung function indices vary with age, sex and ethnicity. This may have resulted in some misdiagnosis and may explain some of the variability in the reported prevalence of obstructive and restrictive defects. A subsidiary aim of this thesis, therefore, was to characterize the nature and prevalence of lung function defects in SCD using classification criteria consistent with current international recommendations [136].
In children

In a study of 37 children with SCD of mean age thirteen (six – eighteen) years (comprising twenty with HbSS, fourteen with HbSC, and three with HbS-β thalassemia disease) and twenty-two control subjects matched for sex, race, and height, Pianosi et al observed a reduction in FEV\textsubscript{1} (88%predicted versus 105%predicted, \( p < 0.0001 \)), VC (89%predicted versus 106%predicted, \( p < 0.001 \)), and TLC (90%predicted versus 100%predicted, \( p < 0.05 \)) in the SCD group compared to the controls [137]. No significant differences were observed between the SCD and control groups for FEV\textsubscript{1}, VC or RV:TLC, suggesting a primarily restrictive abnormality.

A later study by Sylvester et al compared sixty-four children with HbSS disease, of mean age ten (five to sixteen) years, with sixty-four age, sex, and race-matched controls; the SCD children had a significant reduction in FEV\textsubscript{1} (1.5L versus 1.9L, \( p = 0.0008 \)), VC (1.7L versus 2.1L, \( p = 0.001 \)), and FRC measured by helium dilution (1.2L versus 1.3L, \( p = 0.04 \)). Furthermore, the effect of age differed significantly for TLC (\( p = 0.03 \)), VC (\( p = 0.03 \)), and FRC (\( p = 0.01 \)) in the SCD group, for whom these measurements increased more slowly with advancing age compared to the control subjects. These findings are consistent with restrictive abnormalities becoming more prominent with increasing age [138]. Those results contrast with the findings of three other studies. In a retrospective study of twenty infants with SCD of mean age fifteen (three to thirty) months (comprising twelve infants (60%) with HbSS, five (25%) with HbSC, and three (15%) with HbSβ-thalassemia disease), Koumbourlis et al found that patients in the group with HbSS disease and the group
comprising the mixed milder genotypes had high-normal or elevated FRC (112% and 129% predicted) and reduced maximum expiratory flow at FRC ($V'_{\text{max,FRC}}$) (49.8% predicted and 55.7% predicted), suggesting hyperinflation and airflow obstruction [139]. In the HbSS group $R_s$ measured by infant body plethysmography was also elevated (145.3% predicted). Interpretation of these results, however, is limited as no control subjects were tested. The number of individual patients with an FRC above and/or a $V'_{\text{max,FRC}}$ below the limit of normal was not reported, although the authors did state that only three patients (all in the HbSS group) had an FRC below the lower limit of normal, suggesting that restrictive defects were relatively uncommon at this age. Comparing the two groups, the authors found that FRC and $V'_{\text{max,FRC}}$ were lower in the HbSS group ($p < 0.004$ in both cases). Later, Koumbourlis et al carried out a retrospective study of sixty-three children with SCD of mean age eleven (five to eighteen) years. Lung function results were classified as normal, restrictive ($\text{TLC} < \text{LLN}$ and normal $FEV_1:VC$), or obstructive ($FEV_1:VC < \text{LLN}$, and/or the forced expiratory flow between 25% and 75% of vital capacity exhaled ($FEF_{25-75} < \text{LLN}$ with a normal VC) [140]. Spirometry was performed before and after administration of a bronchodilator: a positive response was defined as an increase in $FEV_1$ or $FVC > 10\%$ or in $FEF_{25-75} > 20\%$ from baseline. Twenty-two patients (34%) had an obstructive and five (8%) a restrictive pattern, and twenty-two (34%) had a significant response to bronchodilator (14 of whom were obstructive, two restrictive, and six normal at baseline). Subsequently, Tassel et al retrospectively analysed lung function test results for 184 children with SCD of mean age thirteen (4.8 to 20.0) years from the Creteil SCA Cohort [141] (comprising 159 with HbSS, 17 with HbSC, and eight with HbS-β thalassemia disease) [142].
Lung function results were classified as normal, restrictive (TLC < 80% predicted), or obstructive (FEV$_1$: VC < LLN); a mixed category was not defined. Whilst the definition of obstruction was based on the LLN as recommended by ATS/ERS guidelines [136], the criterion for restriction was based on a fixed percentage-predicted cut-off. Twenty patients (13%) had a restrictive and nine (5%) an obstructive pattern [138]. Spirometry was also repeated after administration of a bronchodilator: a positive response was defined as an increase in FEV$_1$ > 12% with an absolute increase of > 200mls from baseline; a positive response was observed in nine patients (4.9%), two of whom were in the obstructive group. On multiple linear regression, older age and increased leukocyte count were both associated with lower FEV$_1$ (p = 0.0008, p = 0.0003), VC (p = 0.042, p < 0.0001), and TLC (p = 0.001, p = 0.001) respectively i.e. restrictive. The incidence of ACS was not recorded.

The impact of ACS on lung function in children was assessed by Sylvester et al in a cohort of forty children aged six to sixteen years with HbSS disease, twenty of whom had experienced at least one episode of ACS prior to lung function testing [89]. Children with a history of ACS had a significantly higher RV (113%predicted versus 97%predicted, p = 0.003) and total airways resistance measured by body plethysmography (265%predicted versus 194%predicted, p = 0.03) and lower FEV$_1$:VC (95%predicted versus 100%predicted, p =0.04) compared to the non-ACS group, demonstrating an association between ACS and airflow obstruction [89]. Due to the cross-sectional study design, it was not possible to determine if the obstructive abnormalities described preceded or were a consequence of ACS.
episodes. The results of that study highlight a need for a longitudinal study to assess the effect of ACS on the decline of lung function in children with SCD and to determine whether obstructive abnormalities precede ACS episodes.

### 1.7.2 Longitudinal studies

Longitudinal data for pulmonary function in SCD are sparse. Field et al report the only longitudinal study in adults with SCD (aged twenty to sixty years) in a retrospective analysis of lung function test results from the European Haemoglobinopathy Registry [143]. At the final visit, spirometry data were available in ninety-four and static lung volumes in eighty-five patients. Results were classified as normal, restrictive (TLC < LLN), or obstructive (FEV₁:VC < LLN) as per current ATS/ERS guidelines [136]; a mixed category was not included. A restrictive defect was the most common abnormality (35.7%), although patients with obstructive defects were observed (18.5%). Longitudinal analysis was performed for FEV₁ only, using generalised estimating equations to model the trajectory of FEV₁ against age. Raw values, rather the percent predicted, were used for the longitudinal analysis which may have introduced error as normal age-related changes in lung volume would not be taken into account. Subjects included in the longitudinal analysis had between two and four repeated measures over a mean period of thirteen years, but the interval between tests was not reported [143]. FEV₁ declined significantly (p < 0.05) over time at an average rate of 49mls
per year and the authors observed that this was faster than the 20-26ml decline expected in healthy subjects [144]. No association was found between increased rate of pain and a subsequent restrictive (p = 0.97), or obstructive defect (p = 0.09); there was also no association between the occurrence of ACS and the subsequent development of restrictive (p =0.28) or obstructive (p =0.67) defects. Furthermore, no association was found between the rate of decline in FEV₁ and SCD genotype, hereditary persistence of HbF, or smoking history. The effect of ACS on the rate of decline in FEV₁ was not reported.

Two longitudinal studies in children have been reported. In a retrospective analysis, Koumbourlis et al assessed lung function in a cohort of forty-five children with HbSS disease aged 5.4 to 18 years [145]; spirometry and static lung volumes were measured on two occasions with a median time to follow-up of 3.5 years. Lung function results were classified as normal, restrictive (TLC < LLN and normal FEV₁:VC), or obstructive (FEV₁:VC < LLN, and/or the forced expiratory flow between 25% and 75% of vital capacity exhaled (FEF₂₅₋₇₅) < LLN with a normal VC) [140]. At baseline, ten patients (22%) had an obstructive and ten (22%) a restrictive defect. At follow-up, the number of patients with an obstructive defect had increased to twenty (44%), and the number with a restrictive pattern to 12 (27%). A significant decline was observed in FEV₁ (87%predicted to 80%predicted, p < 0.001), VC (84% to 82%, p < 0.05), FEV₁:VC (89% to 85%, p < 0.001), and FEF₂₅₋₇₅(89% to 76%, p < 0.001). No change was seen in TLC, RV, or RV:TLC (p > 0.05). The impact of ACS was not assessed. Those results led the authors to conclude that lung function defects in children with SCD are primarily obstructive and those defects increase in
prevalence and severity over time [145]. A potential flaw in the analysis performed by Koumbourlis *et al* was to attribute longitudinal decline in FEF$_{25-75}$ to increasing airflow obstruction, as this is only valid where the VC does not also change [146, 147]. In a larger retrospective study, MacLean *et al*, analysed spirometry and static lung volumes from a very large cohort (n = 294) of children aged between eight and eighteen years (comprising 112 (38.1%) with HbSS, 49 with HbSC (16.7%), 15 with HbS-beta thalassemia disease (4.8%), one with genotype specified as ‘other’ (0.3%), and 117 (39.8%) in whom genotype data were not available) [148]. All patients included in the final analysis were tested annually on at least two occasions, but the exact data on the number of tests and the time to follow-up were not reported. Lung function results were classified as normal, restrictive (TLC < 70%predicted), or obstructive (FEV$_1$:VC < 80% absolute); a mixed category was not defined. At eight years of age, 2.6% of patients had a restrictive and 0.9%, an obstructive pattern. At seventeen years of age, the prevalence of restrictive defects had increased to 18.7%, and obstructive defects were no longer present. Linear mixed effect modelling (LMM) was used to model the trajectories of lung function indices with increasing age [148]. Results were stratified by sex and a significant decline was observed in FEV$_1$ (2.15%/year for males, p < 0.0001; 2.95%/year for females, p < 0.01), VC (2.64%/year for males, p < 0.0001; 2.36%/year for females, p < 0.0001), FEF$_{25-75}$ (3.42%/year for males, p < 0.0001; 4.10%/year for females, p < 0.0001), and TLC (2.15%/year for males, p < 0.0001; 2.43%/year for females, p < 0.0001). No significant change was seen in FEV$_1$:VC ratio for either sex (p > 0.05). HbSS genotype was associated with a faster decline in FEV$_1$, VC, FEF$_{25-75}$, and TLC compared with HbSC and with a greater likelihood of
developing a restrictive defect (likelihood ratio 9.19, \( p =0.0002 \)). Lower Hb was significantly associated with a lower FEF\(25\% \text{-} 75\%\) at time of testing (\( \beta=0.0031, \ p =0.05 \)). The effect of ACS was not investigated. The authors therefore concluded, in contrast to the study by Koumbourlis et al [145], that lung function in children with SCD is characterised by a progressive decline towards a predominantly restrictive defect. MacLean et al found also a much lower prevalence of lung function defects at baseline and follow-up than Koumbourlis et al (3.5% versus 44% with obstructive or restrictive defects at baseline and 18.7% versus 71% at follow-up). This was particularly pronounced for obstructive defects (22% versus 0.9% at baseline and 44% versus 0% at follow-up). This may in part be because MacLean et al used fixed percentage-predicted cut-offs to define lung function abnormalities. Current American Thoracic Society/European Thoracic Society guidelines recommend that abnormalities are classified according to the lower limit of normal LNN based on percentiles [136], as fixed cut-offs do not take into account the fact that that the limits of the normal range for all lung function indices vary with age, sex and ethnicity. Furthermore, it is not clear from the paper by MacLean et al whether the prevalence of lung function defects was assessed for each patient individually, or predicted from the LMMs derived for TLC and FEV\(_1\):VC. A limitation of the studies [145, 148] is the lack of an appropriate control group; thus it is not possible to determine whether the decline in lung function reported was only seen in SCD patients and hence the magnitude of the effect of SCD. Hence, an aim of this thesis was to examine the rate of decline in lung function in children with SCD in comparison to age and ethnic matched controls and to determine the effect of ACS on any longitudinal change observed.
2. SUMMARY

Sickle cell disease (SCD) is one of the commonest inherited disorders worldwide, affecting an estimated 300 000 new-borns every year. In adulthood, SCD can be associated with multiorgan damage, including pulmonary complications. Acute chest syndrome and sickle chronic lung disease are important sources of morbidity and mortality in children and adults with SCD, and lung function abnormalities are common, even in young children. Acute chest syndrome is associated with poorer lung function and is the most important risk factor for SCLD, for which treatment is only supportive. Asthma and wheezing are associated with an increased risk of acute chest syndrome in children with SCD. The obstructive lung function defects commonly seen in children are often attributed to asthma, but studies have suggested a possible pulmonary vascular origin for airflow obstruction in SCD. Restrictive lung function abnormalities appear to become more prominent with increasing age, but prospective longitudinal studies with appropriate control groups are lacking.
2.1 Hypotheses

- Airway obstruction in SCD may be additionally explained by pulmonary vascular abnormalities resulting from a hyper-dynamic circulation.

- Lung function in SCD children would decline relative to controls particularly in those with asthma or who had suffered ACS episodes.

2.2 Aims

To determine if:

- Lung function abnormalities in adults with SCD were related to pulmonary vascular changes reflecting increased pulmonary vascular volume.

- Increased pulmonary capillary blood volume was related to the airways obstruction seen in SCD children.

- Blood transfusion in SCD children would acutely increase pulmonary capillary blood volume resulting in an increase in airflow obstruction.
• Airway and alveolar nitric oxide production would differ between SCD children and controls and alveolar/airway NO production was related to a hyper-dynamic pulmonary circulation and/or airways obstruction.

Also to:

• To describe the longitudinal changes in lung function in SCD and to assess the impact of acute chest syndrome on the development of lung function abnormalities.
2.3 Methods protocols

2.3.1 Study 1: Pulmonary function, CT and echocardiographic abnormalities in sickle cell disease

Patients with HbSS disease were assessed using pulmonary function tests (spirometry, body plethysmography, transfer factor for carbon monoxide, and impulse oscillometry) together with quantitative high resolution CT-angiography and echocardiography. Lung function abnormalities were documented and the relationship between these abnormalities and interstitial and pulmonary vascular morphological changes was investigated. A subgroup of this cohort had been assessed approximately seven years earlier, and these data were used to investigate longitudinal changes in lung function and to relate these changes to the progression of pulmonary interstitial and vascular disease.

2.3.2 Study 2: Pulmonary capillary blood volume and lung function in children with SCD

Children with HbSS disease, together with age and race-matched healthy control children were assessed using pulmonary function tests (spirometry, body plethysmography, transfer factor for carbon monoxide, and impulse oscillometry). Pulmonary capillary blood volume was measured using the D1NO-D1CO method, and the relationship between lung function and pulmonary capillary blood volume was assessed. A subgroup of the SCD and control children were assessed before
and after administration of a bronchodilator drug to test for reversible airflow obstruction.

2.3.3 Study 3: The acute effects of transfusion on pulmonary capillary blood volume and lung function in children with SCD

Children with HbSS disease who were being treated with regular blood transfusions were assessed using pulmonary function tests (spirometry, body plethysmography, transfer factor for carbon monoxide, and impulse oscillometry) and measurement of pulmonary capillary blood volume using the D_lNO-D_lCO method. Spirometry, transfer factor for carbon monoxide, impulse oscillometry and pulmonary capillary blood volume measurements were repeated immediately following blood transfusion and the acute effects of fluid loading were assessed.

2.3.4 Study 4: Exhaled nitric oxide, lung function, and pulmonary blood flow in children with sickle cell disease

Lung function (spirometry, static lung volumes using whole body plethysmography and gas transfer for carbon monoxide), exhaled nitric oxide (using multiple exhalation flows), alveolar NO production and effective pulmonary blood flow (using an inert gas rebreathing method) were measured in eighteen children with HbSS disease and eighteen healthy age and race-matched controls and the relationships between airway and alveolar nitric oxide production and pulmonary
blood flow and indices of airflow obstruction (FEV₁:VC and respiratory system resistance) were assessed.

2.3.5 Study 5: Longitudinal assessment of lung function in children with sickle cell disease

A prospective longitudinal study was undertaken to examine the rate of decline in lung function, as assessed by spirometry and body plethysmography, in children with HbSS disease in comparison to age and ethnic matched controls. Two cohorts were studied. Cohort one were younger children who were followed for approximately two years, that is at an age when ACS episodes are more common. Cohort two were older and followed for approximately ten years. The impact of ACS and asthma on the rate of decline in lung function was assessed.
3. METHODS

The following techniques were used in all studies:

1. Informed consent.

Written informed consent was obtained from all participants, in the case of adult subjects, or from the participants’ parent(s) or legal guardian(s) in the case of children. Potential participants were contacted by post initially, and after a period of at least two weeks had elapsed, were then approached by the research fellow (A. Lunt), whilst attending their regular clinic appointment at King’s College Hospital, London. Potential participants were given an opportunity to ask questions, and if they were interested in taking part, a provisional appointment to attend the Chest Unit for testing was booked. Participants were telephoned one week before their appointment to confirm they still interested in attending and given a further opportunity to ask any questions. Written consent was obtained at the beginning of the appointment. For children, an age appropriate description of the testing procedure was given, as well as an opportunity to see the equipment used, and to ask questions. Paediatric studies were only conducted with the full support of the child themselves. The studies were approved by the King’s College Hospital Research Ethics Committee under approval numbers LREC 02-0080 and 08/H0808/29.

2. Anthropometry.


5. Measurement of static lung volumes by body plethysmography.

6. Assessment of gas exchange function and estimation of pulmonary capillary blood volume by measurement of the transfer factor for carbon monoxide and nitric oxide.

7. Statistical analysis.
3.1. Anthropometry

Standing height was measured using a wall-mounted Harpenden stadiometer (Holtain Ltd, Dyfed, UK), with a resolution of 0.1cm. The subject was asked to remove any footwear and stand with the back and buttocks against the back-plate of the stadiometer. The feet were positioned such that the heels were in contact with the stadiometer heel plate and the palms were directed medially. The subject was asked to relax the shoulders whilst the head was placed in the Frankfurt plane, such that the inferior margin of the left orbit and the upper margin of the left external auditory meatus was horizontal [149, 150]. When correctly positioned the carriage was lowered until resting on the top of the subject’s head. A small weight was applied to the carriage for stability and the height read from the counter. Weight was measured using a set of electronic scales (Seca, Hamburg, GmbH). The subject was asked to remove footwear and any extraneous clothing. After stepping onto the scales the subject was asked to remain still for the duration of the measurement and the weight recorded to the nearest 0.05kg.

Stadiometer accuracy

The accuracy of the stadiometer was assessed by measurement of three certified calibration rods of length 100, 200, and 250 centimetres respectively (figure 3.1, table 3.1). In each case the error was 0.1% or less and the instrument was linear over the range.
Figure 3-1  Measured height versus rod height for Harpenden stadiometer.

The dashed line is the line of identity.

<table>
<thead>
<tr>
<th>Rod height (cm)</th>
<th>Measured height (cm)</th>
<th>Error (cm)</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>99.9</td>
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<td>-0.10</td>
</tr>
<tr>
<td>150</td>
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<td>0.00</td>
</tr>
<tr>
<td>200</td>
<td>199.9</td>
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<tr>
<td>250</td>
<td>249.8</td>
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<td>-0.08</td>
</tr>
</tbody>
</table>

Table 3-1 Measured height versus rod height for Harpenden stadiometer.
**Weighing scales accuracy**

The accuracy of the weighing scales was assessed using certified calibration weights with a total mass of 15, 30, 45, and 60 kilograms respectively (Figure 3.2, Table 3.2). In each case the error was 1.11% or less and the instrument was linear over the range.

![Figure 3-2 Measured versus applied weight for SECA weighing scales. The dashed line is the line of identity](image-url)
<table>
<thead>
<tr>
<th>Applied weight (kg)</th>
<th>Measured weight (kg)</th>
<th>Error (kg)</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
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<td>-0.78</td>
</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
<td>60</td>
<td>59.91</td>
<td>-0.09</td>
<td>-0.14</td>
</tr>
</tbody>
</table>

Table 3-2 Measured versus applied weight for SECA weighing scales.
3.2. Measurement of pulmonary function

3.2.1. Measurement of respiratory system resistance

Respiratory system resistance was measured using a form of forced oscillation known as impulse oscillometry (IOS). IOS determined respiratory mechanics by superimposing small external pressure signals on the spontaneous breathing pattern of the subject [151]. Since IOS does not involve forced respiratory manoeuvres or deep inspiration, it was unlikely to alter airway dynamics by modification of smooth muscle tone [152]; this was important since the diagnosis of airflow obstruction in reactive airways disease may be missed if substantial bronchodilation takes place as results of deep inspiration [153]. The technique used the external applied pressure signals and their resultant flows to determine lung mechanical parameters. These pressure-flow interactions are largely distinct from the natural respiratory flow parameters and therefore, IOS results are essentially independent of the spontaneous breathing pattern upon which they are superimposed. The physical basis of impulse oscillometry rests on the application of an impulse-shaped multi-frequency external forcing signal to the respiratory flow pattern.

The IOS measuring system is shown in figure 3.3. The head apparatus generated recurrent aperiodic forcing signals of alternating direction. Flow was measured by a Lilly-type heated screen pneumotachograph, with a resistance of 36Pa·s·L⁻¹, a common mode rejection ratio of >60dB for the combination of pneumotachograph
and flow transducer system and a linearity within 2% for flows of <15Ls\(^{-1}\). The proximal side of the pneumotachograph was connected to a pressure transducer. Pressure and flow signals were sampled at 200Hz and digitised by an analogue-digital converter, before post-acquisition digital filtering. The terminal resistor provided a low-impedance pathway for normal respiratory flow, whilst providing a sufficiently high resistance to minimise loss of energy from the superimposed pulses in order that enough impulse pressure is transmitted to the respiratory tract. The range for the device is determined by the value of the terminal resistance and the displacement of the loudspeaker cone and was set according to international recommendations [154, 155]. In this system a terminal resistor of 0.1Pa\(\cdot\)s\(\cdot\)L\(^{-1}\) and a speaker volume displacement of 40ml, accelerated to its endpoint in <40ms yielded peak-peak impulse pressures of around 0.3kPa and acted to minimise contamination of the oscillatory flow and pressure signal by high-order harmonics of the respiratory pattern [156] by ensuring that the generated pressure and flow are sufficiently distant in terms of frequency from the patient’s breathing frequency and providing a forcing signal of sufficient amplitude to ensure adequate signal-to-noise ratio.

The measurement was performed as follows: whilst seated, the subject was asked to breathe room air in a relaxed manner for ninety seconds via the tubing between the mouthpiece and terminating resistor. A rigid mouthpiece with integral tongue guard was used (SpiroGard, Carefusion Ltd., Basingstoke, UK), and the test was performed with the subject’s head in a neutral position, wearing a nose clip, with the cheeks supported by the operators gloved hands. A bacterial filter was used,
with an automated correction for the additional resistance. The loudspeaker transmitted short-duration pressure impulses via the Y-adaptor, pneumotachograph and mouthpiece into the respiratory tract. The pneumotachograph and pressure transducer registered the composite signals containing breathing activity and the forcing impulse signal, which were acquired for further processing using Fourier analysis by propriety software (JLAB version 5.4 Carefusion Ltd., Basingstoke, UK). A coherence value of at least 0.9 across the frequency range was required for a technically acceptable measurement.

![Figure 3-3 Schematic of impulse oscillometry system.](image)

Loudspeaker enclosure at top, connected to a Y-adaptor at one upper arm, an exit for flow with terminal resistor at the second upper arm and a lower arm connected to the pneumotachograph. Pulsed flows produced by the loudspeaker are shown as a lightly shaded thick line, a component of which exits through the terminal resistor and a component of which passes through the pneumotachograph and mouthpiece.
The subject's spontaneous respiratory flow is shown as a shaded thick line from the mouthpiece through the Y-connector, exiting through the terminal resistor. Adapted from Oostveen et al [162].

The indices of respiratory mechanics provided by IOS were derived from the respiratory system impedance ($Z_{rs}$). Flow and pressure channels characterised both the underlying respiratory flow and pressure, and the superimposed forced oscillation signals. $Z_{rs}$ was defined as the ratio of effective pressure ($P_{rs}$) and effective flow ($V'_{rs}$) derived from the superimposed forced oscillations, after being separated from the underlying respiratory pressure and flow and their harmonics, at each frequency present in the forced oscillation signal. IOS is a multi-frequency oscillation method and provided measures of respiratory mechanical properties in terms of $Z_{rs}$, as a function of frequency ($f$), hence allowing the recognition of characteristic respiratory responses at different oscillation frequencies.

**Respiratory Resistance**

$Z_{rs}$ is the complex sum of lung resistance and reactance and expresses the overall impedance to airflow in the lung. Respiratory system resistance ($R_{rs}$) is the component of lung impedance associated with frictional loss in both the airways and the lung parenchyma and represents the component of transpulmonary pressure that is in-phase with flow. $R_{rs}$ is the sum of viscous resistances within the lung and therefore includes proximal and distal airways, lung tissue and chest wall resistance. In general, airway resistance dominates, while lung tissue and chest...
wall resistance are usually negligible [157]. Rs was recorded over a frequency range of five to twenty Hertz and frequency-specific Rs values were obtained using a Fourier decomposition of the Rs signal. Impulse oscillometry was used to measure airways resistance because, whilst the results are comparable to those obtained by body plethysmography, the requirements for patient cooperation are less stringent (e.g., cheek support can be provided by the operator, rather than the patient) [158]. Furthermore, the multi-frequency nature of IOS provides additional information regarding the anatomical site of airflow obstruction. Collection and analysis of IOS data also forms part on an ongoing departmental data collection effort aimed at characterizing IOS parameters in various patient groups (including community measurements, where body plethysmography is not feasible)

**Accuracy of the IOS pneumotachograph**

The accuracy of the IOS system pneumotachograph was assessed by comparing the measured volume to the certified volume supplied by calibration syringes with a stroke-volume of 1, 2, 3, 4, 5, 6, and 7 litres. Three strokes were applied manually from each syringe using a steady motion and the mean value plotted (figure 3.4, table 3.3). In each case the error was 1.29% or less and the instrument was linear over the range.
Figure 3-4 Measured volume versus syringe volume for the IOS system pneumotachograph.

The dashed line is the line of identity.

<table>
<thead>
<tr>
<th>Syringe volume (L)</th>
<th>Measured volume (L)</th>
<th>Error (L)</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<tr>
<td>7</td>
<td>7.09</td>
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<td>1.29</td>
</tr>
</tbody>
</table>

Table 3-3 Measured volume versus syringe volume for the IOS system pneumotachograph.
3.2.2. Spirometry

The forced expiratory volume in one second (FEV₁), forced vital capacity (FVC) and peak expiratory flow (PEF) were measured using a Jaeger Masterscreen PFT system (Carefusion Ltd., Basingstoke, UK). Flow was measured by a Lilly-type heated screen pneumotachograph and volume measurements were derived using a software-based integrator (JLAB software version 5.4).

Spirometry was performed according to ATS/ERS guidelines [159]. The technique was explained to the subjects before commencing testing. The subject was seated wearing a nose-clip and asked to breathe normally through the pneumotachograph via a mouthpiece. The subject then performed a slow inspiration to TLC followed by a maximal forced expiration to RV, as hard and as fast as possible. Verbal encouragement was given to expire for as long as possible and to refrain from breathing in again until instructed. The system allowed the flow-volume and volume-time traces to be visualised in real-time to determine when expiratory flow had ceased and the manoeuvre could be terminated. Any traces showing air leak, incomplete inspiration, a slow rise to PEF, cough, or early termination of expiration were rejected. A minimum of three attempts were made, and testing continued until two manoeuvres with an FEV₁ and FVC within 5% of each other were recorded. For each subject, spirometry was performed after lung volume measurements and measurement of airway mechanics to avoid changes in lung volume from spirometrically-induced bronchoconstriction and changes in airway function due to altered bronchomotor tone following deep inspiration.
**Accuracy of the spirometer pneumotachograph**

The accuracy of the spirometer pneumotachograph was assessed by comparing the measured volume to the certified volume supplied by calibration syringes with a stroke-volume of 1, 2, 3, 4, 5, 6, and 7 litres. Three strokes were applied manually from each syringe using a steady motion and the mean value plotted (figure 3.5, table 3.4). In each case the error was 1.00% or less and the instrument was linear over the range.

![Figure 3-5 Measured volume versus syringe volume for the PFT system pneumotachograph. The dashed line is the line of identity.](image-url)
<table>
<thead>
<tr>
<th>Syringe volume (L)</th>
<th>Measured volume (L)</th>
<th>Error (L)</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>2.00</td>
<td>0.00</td>
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</tr>
<tr>
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<td>0.00</td>
</tr>
<tr>
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<td>3.96</td>
<td>-0.04</td>
<td>-1.00</td>
</tr>
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<td>5.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>6</td>
<td>5.99</td>
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<tr>
<td>7</td>
<td>7.04</td>
<td>0.04</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Table 3-4 Measured volume versus syringe volume for the PFT system pneumotachograph.
3.2.3. **Body plethysmography**

Total lung capacity ($TLC_{\text{pleth}}$), residual volume ($RV_{\text{pleth}}$), and functional residual capacity ($FRC_{\text{pleth}}$) were measured using a constant volume 850 litre body plethysmograph incorporating a Lilly-type heated screen pneumotachograph (Carefusion Ltd., Basingstoke, UK). Calibration of the plethysmograph pneumotachograph and mouth pressure and box pressure transducers was performed prior to each testing session using the propriety calibration software (JLAB version 5.4). Barometric pressure, temperature, and humidity were recorded automatically by an external sensor array. The pneumotachograph was calibrated using a certified calibration syringe of 3L volume (Hans Rudolph Inc, Kansas City, USA). Pressure transducers were calibrated using an integral sinusoidal pump with a stroke volume of 50ml and automatic calibration software (JLAB version 5.4).

Measurements were performed according to ATS/ERS guidelines [160]. The subject was asked to sit in the plethysmograph for at least two minutes to allow equilibration of the temperature and pressure within the box. The subject was then requested to breathe normally through the mouthpiece for a period of 30 – 60 seconds. After the period of quiet breathing and at end-expiration, the mouthpiece was occluded by a shutter mechanism mounted at the distal end of the pneumotachograph. The subject made inspiratory and expiratory efforts against the occlusion, during which time measurements of box and mouth pressure were recorded. The shutter remained closed for a fixed duration of three seconds, after
which time the subject was instructed to make a maximal expiratory effort to residual volume. The breathing trace was monitored on the visual display and when expiratory flow had ceased, the subject was instructed to perform a maximal inspiratory effort to TLC. Static lung volumes were calculated from Boyle’s Law which states that, if temperature is constant, the volume of a given mass of gas is inversely proportional to its pressure. During the inspiratory efforts against the occluded mouthpiece, there was a resultant decrease in alveolar pressure and a corresponding increase in lung volumes. Boyle’s law can therefore be rewritten as:

\[ P_1V_i = (P_i - \Delta P)(V_i + \Delta V) \]

Where:

\[ V_1 = \text{the initial volume of the lungs (equivalent to } FRC_{pleth}) \]

\[ P_1 = \text{barometric pressure} \]

\[ \Delta V = \text{the change in lung volume during a respiratory effort} \]

\[ \Delta P = \text{the change in alveolar pressure corresponding to } \Delta V \]

Therefore, multiplying out:

\[ P_1V_i = P_iV_i + P_i\Delta V - V_i\Delta P - \Delta P\Delta V \]

But the product \( \Delta P\Delta V \) is negligible therefore the equation simplifies to:

\[ V_i\Delta P = P_i\Delta V \]

Rearranging yields:
\[ V_1 = P_1 \cdot \left( \frac{\Delta V}{\Delta P} \right) \equiv FRC_{pleth} \]

The change in lung volume was derived from the change in box pressure during the manoeuvre, and the relationship between changes in box pressure and volume was determined during the sinusoidal calibration. Mouth pressure was assumed to accurately reflect alveolar pressure during the occlusion when there was zero flow. The flow during the vital capacity (VC) manoeuvre was recorded by the pneumotachograph, and integrated to yield volume. From this the expiratory reserve volume (ERV) was determined, and used to derive \( RV_{pleth} \) and \( TLC_{pleth} \) as follows:

\[
RV_{pleth} = FRC_{pleth} - ERV \\
TLC_{pleth} = RV_{pleth} + VC
\]

The mean value of three measurements within <10% variation was reported [161].

**Accuracy of the body plethysmograph pneumotachograph**

The accuracy of the spirometer plethysmograph pneumotachograph was assessed by comparing the measured volume to the certified volume supplied by calibration syringes with a stroke-volume of 1, 2, 3, 4, 5, 6, and 7 litres. Three strokes were applied manually from each syringe using a steady motion and the mean value
plotted (figure 3.6, table 3.5). In each case the error was 1.71% or less and the instrument was linear over the range.

Figure 3-6 Measured volume versus syringe volume for the body plethysmograph pneumotachograph.

The dashed line is the line of identity.

<table>
<thead>
<tr>
<th>Syringe volume (L)</th>
<th>Measured volume (L)</th>
<th>Error (L)</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>1.99</td>
<td>-0.01</td>
<td>-0.50</td>
</tr>
<tr>
<td>3</td>
<td>2.98</td>
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<td>-0.67</td>
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<tr>
<td>4</td>
<td>4.04</td>
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<td>1.00</td>
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<td>0.05</td>
<td>1.00</td>
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<td>6</td>
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<tr>
<td>7</td>
<td>7.12</td>
<td>0.12</td>
<td>1.71</td>
</tr>
</tbody>
</table>

Table 3-5 Measured volume versus syringe volume for the body plethysmograph pneumotachograph.
3.2.4. Lung gas transfer

Total lung transfer factor for carbon monoxide ($D_{L}CO$), alveolar volume ($V_A$), and gas transfer for carbon monoxide per unit lung volume ($KCO$) were measured using the single-breath breath-hold method [161]. The system comprised a katharometer-based helium analyser, and an infrared analyser for carbon monoxide (Masterscreen PFT, Carefusion Ltd., Basingstoke, UK). Flow was measured by a Lilly-type heated screen pneumotachograph and volume measurements were derived using a software-based integrator (JLAB software version 5.3). The katharometer detected changes in helium concentration by relating it to changes in thermal conductivity, which is related inversely to the molecular weight of a gas. The katharometer consists of two parallel tubes with integral heating elements arranged in a bridge circuit configuration so that resistance changes due to unequal cooling by the gases in each tube could be detected. One tube received a reference gas, while the second tube received the gas mixture to be analysed. The infrared analyser comprised two parallel chambers mounted between an infrared light source and an infrared receiver. A reference gas was passed through the first chamber and the sample gas fed into the second. The difference in absorption of infrared energy between the reference and sample gases allowed the concentration of carbon monoxide to be determined. The gas analysers and pneumotachograph were calibrated prior to each testing session. Katharometer calibration was performed using certified test gas containing 9% helium; whilst the infrared analyser was with a certified cylinder containing 9%He and 0.28% carbon monoxide. The pneumotachograph was calibrated using a
certified calibration syringe of 3L volume (Hans Rudolph Inc., Kansas City, USA). The subject was instructed in the technique required prior to testing. Wearing a nose-clip, the subject was seated and asked to breathe normally through the test circuit via a flanged mouthpiece attached to the pneumotachograph. The subject was then asked to inspire maximally to TLC, expire maximally to RV, and then perform a second maximal inspiration to TLC. The test gas (9%He, 0.28%C0, 19%O2 and balance N2) was automatically introduced at the start of the second maximal inspiration. The subject was instructed to hold their breath for 10 seconds at TLC, whilst the volume trace was monitored for leaks or Valsalva efforts. A shutter was automatically closed at the end of maximal inspiration to occlude the mouthpiece. After 10 seconds the shutter was released and the subject instructed to exhale to RV. The first 750ml, representing a bronchial sample, was discarded, and the following 750ml, the alveolar sample, was retained for analysis. If the subject’s VC was less than 1.5L the discard volume was reduced to 600ml.

\[ \text{D}_{1\text{CO}}, \text{V}_{A}, \text{and KCO were calculated from the change in CO and He concentrations as follows:} \]

\[ \text{VA} = \left( \text{V}_{in} - \text{V}_{ds} \right) \left( \frac{\text{He}_{init}}{\text{He}_{final}} \right) \]

Where:

\[ \text{V}_{in} = \text{inspiratory VC during manoeuvre} \]

\[ \text{V}_{ds} = \text{total deadspace, comprising anatomical and equipment deadspace} \]

\[ \text{He}_{init} = \text{initial concentration of helium in test gas} \]
He_{final} = the helium concentration in the expired alveolar sample

Due to dilution of the test gas by the residual volume, the alveolar starting concentration of CO is not equivalent to the cylinder concentration, but may be calculated from the dilution of the helium component of the test gas as follows:

\[ CO_{alv(t=0)} = CO_{cyl} \cdot \left( \frac{He_{init}}{He_{final}} \right) \]

Where:

- \( CO_{alv(t=0)} = \) the starting concentration of CO in the lung prior to gas exchange
- \( CO_{cyl} = \) the nominal CO concentration in the test gas

The breath-hold time was defined according to the system of Jones and Meade [162]. The time for gas exchange was defined as the period between the time at one-third of the inspiratory volume (\([CO] = CO_{alv(t=0)}\)) and one half of the total expired volume after breath-holding (\([CO] = CO_{alv(t=10)}\)). By taking the slope defined by \( \ln(CO_{alv(t=0)}) \) and \( \ln(CO_{alv(t=10)}) \), the gradient of the CO uptake curve over the contact time was derived. This is the rate constant for CO uptake, which is equivalent to \( k_{CO} \). D_{l,CO} is then derived from:
With:

\[
K_{CO} = \frac{D_{L}CO}{V_{A}}
\]

A minimum of two tests were performed, with the mean value of two results within 10% of each other reported. A maximum of five attempts were made, with an interval of four minutes between each test run in order to ensure wash-out of helium [161].

The reported result was adjusted for haemoglobin concentration [163], using a blood test performed within six weeks of the test date as part of the patient’s routine clinical management when clinically stable, using the following formula:

\[
D_{L}CO_{c} = D_{L}CO_{\text{obs}} \cdot \frac{10.22 + \left[H_{b}\right]}{1.7 \cdot \left[H_{b}\right]}
\]

Where \(D_{L}CO_{c}\) is the gas transfer corrected for haemoglobin concentration.
**Pulmonary capillary blood volume**

Prior to testing, nitric oxide (NO) was added to the test gas mix to a concentration of 40ppm, which allowed the transfer factor for nitric oxide ($D_L NO$) to be assessed simultaneously with $D_L CO$ using the same procedure described above. NO concentration was measured using an electrochemical sensor incorporated into the gas analyser system. The method of Borland *et al* [164] was used to estimate pulmonary capillary blood volume as follows. According to the equation of Roughton and Forster, the $D_L CO$ is described by the following relationship:

\[
\frac{1}{D_L CO} = \frac{1}{D_m CO} + \frac{1}{\Theta CO \cdot V_c}.
\]

Where:

$D_m CO =$ the pulmonary membrane diffusing capacity for carbon monoxide (equivalent to the carbon monoxide conductance across the alveolar-capillary tissue membrane and plasma barrier)

$\Theta CO =$ the rate of carbon monoxide uptake by whole blood and combination with haemoglobin

$V_c =$ the pulmonary capillary blood volume.

The same relationship applies to the $D_L NO$, but since the reaction rate of NO binding with haemoglobin is approximately 280 times faster than that of CO, the
rate of NO uptake by the blood ($\Theta_{CO}$) is extremely large and therefore the term $1/(\Theta_{CO}V_c)$ becomes very small and may be neglected, yielding:

$$D_L NO = D_m NO.$$ 

But, by Graham's Law [165]:

$$\frac{D_L NO}{D_m CO} = \frac{D_m NO}{D_m CO} \frac{\alpha NO}{\alpha CO} = \frac{MW_{CO}}{MW_{NO}} = 1.93,$$

and therefore $D_m CO = 1.93/D_m NO$ [166].

Where:

MW$_{CO}$ = the molecular weight of CO = 28

MW$_{NO}$ = the molecular weight of NO = 30

$\alpha CO$ = the solubility factor of CO in water at 37.5°C = 0.0183 ml$^{-1}$atm$^{-1}$

$\alpha NO$ = the solubility factor of NO in water at 37.5°C = 0.0364 ml$^{-1}$atm$^{-1}$

$D_m CO$ was then substituted back into the Roughton-Forster equation which was then rearranged to obtain $V_c$ [167] as:
\[ V_c = \frac{1}{\frac{1}{D_L CO} - \frac{1}{D_m CO}} \cdot \theta_{CO} \]

**Accuracy of the gas transfer system pneumotachograph**

The gas transfer system used the same pneumotachograph used to perform spirometry. The accuracy of this device is assessed in section 3.2.2 (figure 3.5, table 3.4).

**Accuracy of the gas transfer system gas analysers**

The accuracy of the Masterscreen PFT gas analysers was checked using a gas dilution method. A certified calibration syringe of known volume was used as a ‘simulated’ patient, and the DLCO test was performed as described above, with the stroke volume set to half of the syringe volume. Since the system was sealed and there was no gas uptake by the ‘patient’, the reduction in CO and Helium concentrations at the end of the test was solely due to dilution by the syringe volume. The dilution ratio was then used to calculate the syringe volume by:
\[ V_{\text{mean}} = (V_{\text{stroke}} - V_{ds}) \cdot \left( \frac{\text{Conc}_{\text{init}}}{\text{Conc}_{\text{final}}} \right). \]

\( V_{\text{stroke}} = \) syringe stroke volume

\( V_{ds} = \) system deadspace

\( \text{Conc}_{\text{init}} = \) initial concentration of helium or CO

\( \text{Conc}_{\text{final}} = \) initial concentration of helium or CO after manoeuvre

The difference between the measured and certified syringe volumes is the analyser error. Testing was performed with syringe volumes of 2, 3, 4, 5, 6, and 7 litres; in each case the error was 4.6% or less and the analysers were linear over the range (figures 3.7, 3.8 and 3.9, tables 3.6, 3.7 and 3.8).
Figure 3-7 Measured volume by gas dilution versus syringe volume for the helium analyser.

The dashed line is the line of identity.

<table>
<thead>
<tr>
<th>Syringe volume (L)</th>
<th>Measured volume (L)</th>
<th>Error (L)</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2.06</td>
<td>0.06</td>
<td>2.78</td>
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<tr>
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<td>2.91</td>
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<td>4.08</td>
<td>0.08</td>
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<td>5.07</td>
<td>0.07</td>
<td>1.38</td>
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<td>6.13</td>
<td>0.13</td>
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<tr>
<td>7</td>
<td>6.92</td>
<td>-0.08</td>
<td>-1.16</td>
</tr>
</tbody>
</table>

Table 3-6 Measured volume by gas dilution versus syringe volume for the helium analyser.
Figure 3-8 Measured volume by gas dilution versus syringe volume for the CO analyser.

The dashed line is the line of identity

<table>
<thead>
<tr>
<th>Syringe volume (L)</th>
<th>Measured volume (L)</th>
<th>Error (L)</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1.97</td>
<td>-0.03</td>
<td>-1.27</td>
</tr>
<tr>
<td>3</td>
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<td>-0.51</td>
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<td>-0.02</td>
<td>-0.39</td>
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<td>-4.60</td>
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<td>7</td>
<td>6.87</td>
<td>-0.13</td>
<td>-1.84</td>
</tr>
</tbody>
</table>

Table 3-7 Measured volume by gas dilution versus syringe volume for the CO analyser.
Figure 3-9 Measured volume by gas dilution versus syringe volume for the NO analyser.

The dashed line is the line of identity

<table>
<thead>
<tr>
<th>Syringe volume (L)</th>
<th>Measured volume (L)</th>
<th>Error (L)</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1.92</td>
<td>-0.08</td>
<td>-3.77</td>
</tr>
<tr>
<td>3</td>
<td>3.13</td>
<td>0.13</td>
<td>4.21</td>
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<td>0.11</td>
<td>2.70</td>
</tr>
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<td>-2.54</td>
</tr>
<tr>
<td>6</td>
<td>5.92</td>
<td>-0.08</td>
<td>-1.28</td>
</tr>
<tr>
<td>7</td>
<td>7.04</td>
<td>0.04</td>
<td>0.58</td>
</tr>
</tbody>
</table>

Table 3-8 Measured volume by gas dilution versus syringe volume for the NO analyser.
3.2.5. Multiple-flow exhaled nitric oxide

Exhaled nitric oxide was assessed according to American Thoracic Society/European Thoracic Society criteria [168] using a commercial online NO analyser system (Hypair feNO, Medisoft Cardio-respiratory Instrumentation, Sorinnes, Belgium) employing an electrochemical fuel-cell NO sensor. Whilst seated comfortably, the subject was asked to insert the mouthpiece connected to the analyser circuit and inhale to total lung capacity over a period of two to three seconds. During inspiration, the breathing circuit was switched through an NO-scrubbing filter to prevent contamination of the expired sample by ambient NO in the inspired gas. A nose clip was not worn to avoid accumulation and leakage of nasal NO from the posterior nasopharynx [169].

Upon reaching TLC, subject was asked to exhale steadily into the breathing circuit. A constant expiratory flow rate was achieved by exhaling against a fixed resistor with real-time visual feedback to maintain the expiratory flow within 10% of the desired value for duration of the measurement phase. Mouth pressure was maintained at 16cmH$_2$O throughout the exhalation to ensure velum closure and minimise contamination of the expired sample with NO of nasal origin [170]. The exhalation was maintained for at least ten seconds to ensure washout of the airway compartment, and NO level was monitored continuously to ensure that a plateau had been reached. The plateau concentration of NO ($f_{eNO}$) was evaluated over a three-second window of the exhalation profile such that the difference in NO concentration at the start and end of the window was less than 10%, and at no
time during the three second window did the NO concentration differ from the value at the start or end by more than 10%. $F_{eNO}$ was defined as the mean NO concentration over the three-second window. Measurements with evidence of cough, air leak, nasal contamination, or failure to maintain constant flow were discarded. Measurements were performed at expiratory flows of 50, 100, 150 and 350ml/s, and at each flow rate $f_{eNO}$ was reported as the mean of three technically acceptable measurements within 10% of each other.

**Derivation of alveolar and airway NO**

The trumpet-model with axial diffusion (TMAD) developed by Condorelli et al was used to estimate alveolar NO concentration ($C_{A,NO}$) and maximal airway NO flux ($J'_{aw,NO}$) from multi-flow $f_{eNO}$ measurements [171]. The model assumes that a plateau concentration ($F_{eNO}$) is reached in the exhaled air measured at the mouth and that during the constant-flow exhalation manoeuvres the concentration and flux of NO in the distal (alveolar) and proximal (airway) zones of the lung approach steady-state (and are therefore independent of time), which is valid for flow rates greater than approximately 50ml/s [171-173]. Under these circumstances the model yields the following relationship:

$$\dot{V}_{NO} = (C_{A,NO} + J'_{aw,NO} \cdot 0.00078)\dot{V} + \frac{J'_{aw,NO}}{1.7}$$
Where $\dot{V}_{NO}$ is the rate of NO exhaled, which is given by the product of NO concentration in nanolitres per litre and expiratory flow rate in litres per minute (BTPS). Thus a plot of $\dot{V}_{NO}$ versus exhaled flow produces a linear relationship in which the slope is equal to $C_{A,NO} + J'_{aw,NO} \cdot 0.00078$ and the intercept is equal to $J'_{aw,NO}/1.7$. Therefore, $C_{A,NO}$ and $J'_{aw,NO}$ were estimated from a plot of $\dot{V}_{NO}$ versus exhaled flow using the following relationships:

\[
C_{A,NO} = S - I \left( \frac{0.00078 \text{Sl}}{0.57} \right) = S - \frac{I}{740\text{ml}/s} 
\]

\[
J'_{aw,NO} = 1.7 \cdot I 
\]

Where $S$ was the slope and $I$ was the y-intercept obtained using simple linear regression (figure 3.10).
Figure 3-10 Illustrative data demonstrating the rate of NO exhalation vs. flow technique to estimate mean alveolar concentration and maximum airway NO flux.

**Accuracy of the exhaled NO system flow sensor**

The accuracy of the Hypair FeNO system flow sensor was assessed by comparing the measured volume to the certified volume supplied by calibration syringes with a stroke-volume of 1, 2, 3, 4, 5, 6, and 7 litres. Three strokes were applied manually from each syringe using a steady motion and the mean value plotted (figure 3.11, table). In each case the error was 2.8% or less and the device was linear across the range.
Figure 3-11: Measured volume versus syringe volume for the Hypair FE\textsubscript{NO} system flow sensor.

The dashed line is the line of identity.

<table>
<thead>
<tr>
<th>Syringe volume (L)</th>
<th>Measured volume (L)</th>
<th>Error (L)</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.03</td>
<td>0.03</td>
<td>2.81</td>
</tr>
<tr>
<td>2</td>
<td>1.99</td>
<td>-0.01</td>
<td>-0.71</td>
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<tr>
<td>3</td>
<td>2.97</td>
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<td>-0.87</td>
</tr>
<tr>
<td>4</td>
<td>4.01</td>
<td>0.01</td>
<td>0.15</td>
</tr>
<tr>
<td>5</td>
<td>4.97</td>
<td>-0.03</td>
<td>-0.65</td>
</tr>
<tr>
<td>6</td>
<td>5.94</td>
<td>-0.06</td>
<td>-0.94</td>
</tr>
<tr>
<td>7</td>
<td>7.01</td>
<td>0.01</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Table 3-9 Measured volume versus syringe volume for the Hypair FE\textsubscript{NO} system flow sensor.
Accuracy of the exhaled NO system gas analyser

The accuracy of the electrochemical NO analyser cell was assessed using certified calibration gas cylinders with NO concentrations of 0, 33, 66, and 112 ppb. Each cylinder was connected to the analyser in turn using a dedicated calibration inlet valve and the measured values displayed. In each case the error was less than 2 ppb and the analyser was linear across the range (Figure 3.12, table 3.10).

Figure 3-12 Measured versus cylinder NO concentrations for the Hypair FE_{NO} analyser.

The dashed line is the line of identity
<table>
<thead>
<tr>
<th>Cylinder NO concentration (ppb)</th>
<th>Measured NO concentration (ppb)</th>
<th>Error (ppb)</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>33</td>
<td>34.2</td>
<td>1.2</td>
<td>3.64</td>
</tr>
<tr>
<td>67</td>
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<td>1.6</td>
<td>2.39</td>
</tr>
<tr>
<td>112</td>
<td>112.5</td>
<td>0.5</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Table 3-10 Measured versus cylinder NO concentrations for the Hypair FE<sub>NO</sub> analyser.
3.2.6. Pulmonary blood flow

Effective pulmonary blood flow was measured by the inert-gas rebreathing method using a commercially available system (Innocor, Innovision ApS, Glamsbjerg, Denmark). The system used an inert soluble gas (0.5% nitrous oxide), an inert insoluble gas (0.1% sulphur hexafluoride) and 28% oxygen in N₂. Wearing a nose clip, rebreathing was performed from a 2–4 litre rubber bag and gases were sampled continuously at the mouth and passed to a rapid photo-acoustic spectrograph analyser within the Innocor unit [174]. Respiratory flow was measured during the rebreathing manoeuvre by an integrated Lily-type heated pneumotachograph. The volume to which the bag was filled with the test gas was adjusted to 60% of the subjects’ vital capacity. Rebreathing was performed over a thirty second period and subjects were trained to follow a graphical tachometer on the Innocor display screen and verbal prompts in order to ensure a constant respiratory rate of 20 breaths/min. Subjects were instructed to empty the rebreathing bag fully with each breath in order to ensure a constant ventilation volume. The pulmonary blood flow (Q_{pulm}) was calculated from the rate of uptake of nitrous oxide into the blood as described below. 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 [175].

Calculation of pulmonary blood flow was based on single-compartment lung model which assumed that pulmonary blood flow was constant and mixed venous
concentration of the soluble gas was negligible during the manoeuvre, and that complete mixing of all gases in the volume comprising the lung and bag volume takes place.

Before calculating pulmonary blood flow, the total systemic volume ($V_{s,tot}$) of the lung-rebreathing circuit system was calculated, which comprises:

$$V_{s,tot} = V_L + V_{ds} + V_{ds,rb} + V_{rb}.$$  

Where:

$V_L$ = Lung volume at end-expiration during the rebreathing manoeuvre

$V_{ds}$ = Dead space volume of the rebreathing valve (RBU)

$V_{ds,rb}$ = Residual volume of the rebreathing bag when empty

$V_{rb}$ = Volume of rebreathing bag

During the rebreathing period the concentration of insoluble gas (SF$_6$) decreased from the initial value in the bag (denoted $F_{i0}$) until equilibration had taken place and the concentration remained constant (denoted $F_{i,eq}$). The single-compartment model uses the total systemic volume at time zero (denoted $t_0$), where zero is defined as the middle of the first inspiration and before shrinkage has taken place due to oxygen uptake. This volume is, however, difficult to measure as the gas dilution technique requires the insoluble gas to be fully mixed which is not the case.
at time zero and therefore a back-extrapolation method was used to provide an estimate. Least-squares linear regression was used to fit a straight line through the end-expiratory points of the insoluble gas dilution curve where adequate mixing was obtained. The insoluble gas concentration at time zero was then determined as the point where the extrapolated line crossed time zero (figure 3.13).

![Figure 3-13 Insoluble gas concentration during rebreathing](image)

Then total systemic volume at time zero was then calculated as:

\[ V_{s,\text{tot}} = \frac{F_{i,0}}{F_{i,\text{eq}}} \cdot V_{rb} \]

Where:

\( V_{s,\text{tot}} = \text{Total systemic volume} \)
\( F_i^0 \) = Initial concentration of insoluble gas in the rebreathing bag

\( F_{i,eq} \) = Equilibrium concentration of insoluble gas (back extrapolated to \( t = 0 \))

\( V_{rb} \) = Rebreathing bag volume

Pulmonary blood flow was derived from the rate of disappearance of the soluble gas (NO\(_2\)) during the rebreathing manoeuvre. Assuming a constant \( Q_{pulm} \), the disappearance curve describes a monotonically decreasing exponential function of time, where the rate constant is proportional to \( Q_{pulm} \). By plotting on semi-logarithmic axes, a linear decay function was obtained, where the slope (\( \beta \)) was determined by fitting a least-squares linear regression line through the expiratory points of the logarithmic soluble gas concentration (figure 3.14).
Figure 3-14: Semi logarithmic plot of soluble gas concentration during the rebreathing manoeuvre. The slope of the regression line is proportional $Q_{\text{pulm}}$.

So:

$$
\beta = \frac{\ln(F_{s,2}') - \ln(F_{s,1}')}{T_2 - T_1}.
$$

The pulmonary blood flow was then calculated from:

$$
Q_{\text{pulm}} = -\beta \cdot \frac{V_{\text{s,tot}} \cdot C_1 + C_2}{\alpha_b}.
$$
Where:

\( Q_{\text{pulm}} = \) Pulmonary blood flow

\( \beta = \) least-squares linear regression line through the expiratory points of the logarithm of the soluble gas concentration

\( V_{s,\text{tot}} = \) Total systemic volume

\( C_1 = \) Constant to correct for barometric pressure = \( 760/(P_B - 47) \) mmHg

\( C_2 = \alpha_t \cdot V_t = \) Constant to correct for the absorption of soluble gas into lung tissue

\( \alpha_b = \) Bunsen solubility coefficient in blood [ml STPD/ml/atm @ 37°C] (\( \alpha_b, \) N\(_2\)O = 0.412)

\( \alpha_t = \) Bunsen solubility coefficient in blood [ml STPD/ml/atm @ 37°C] (\( \alpha_t, \) N\(_2\)O = 0.407)

\( P_B = \) Ambient barometric pressure in mmHg
**Accuracy of the Innocor pneumotachograph**

The accuracy of the Innocor pneumotachograph was assessed by comparing the measured volume to the certified volume supplied by calibration syringes with a stroke-volume of 1, 2, 3, 4, 5, 6, and 7 litres. Three strokes were applied manually from each syringe using a steady motion and the mean value plotted (Figure 3.15, table 3.11). In each case the error was 2.85% or less and the device was linear across the range.

![Graph](Image)

**Figure 3-15 Measured volume versus syringe volume for the Innocor pneumotachograph.**

The dashed line is the line of identity.
### Table 3-11 Measured volume versus syringe volume for the Innocor pneumotachograph

<table>
<thead>
<tr>
<th>Syringe volume (L)</th>
<th>Measured volume (L)</th>
<th>Error (L)</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.98</td>
<td>0.02</td>
<td>-2.24</td>
</tr>
<tr>
<td>2</td>
<td>2.06</td>
<td>0.06</td>
<td>2.85</td>
</tr>
<tr>
<td>3</td>
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<td>-0.89</td>
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<tr>
<td>4</td>
<td>4.05</td>
<td>0.05</td>
<td>1.34</td>
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<tr>
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</tr>
<tr>
<td>6</td>
<td>5.98</td>
<td>-0.02</td>
<td>-0.27</td>
</tr>
<tr>
<td>7</td>
<td>6.93</td>
<td>-0.07</td>
<td>-1.03</td>
</tr>
</tbody>
</table>

**Accuracy of the Innocor photo-acoustic spectrograph gas analyser**

The accuracy of the Masterscreen PFT gas analysers was checked using a gas dilution method as described in section 3.3.4 for the gas transfer system gas analysers. A certified calibration syringe of known volume was used as a ‘simulated’ patient, and the inert gas rebreathing test was performed as described above, with the stroke volume set to half of the syringe volume. Testing was performed with syringe volumes of 2, 3, 4, 5, 6, and 7 litres; in each case the error was 0.94% or less and the analysers were linear over the range (figure 3.16 and 3.17, table 3.12 and 3.13).
Figure 3.16 Measured volume by gas dilution versus syringe volume for the Innocor SF₆ analyser.

The dashed line is the line of identity.

<table>
<thead>
<tr>
<th>Syringe volume (L)</th>
<th>Measured volume (L)</th>
<th>Error (L)</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
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<td>0.01</td>
<td>0.60</td>
</tr>
<tr>
<td>3</td>
<td>2.97</td>
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<td>-0.94</td>
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<td>0.47</td>
</tr>
<tr>
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<td>5.01</td>
<td>0.01</td>
<td>0.12</td>
</tr>
<tr>
<td>6</td>
<td>6.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>7</td>
<td>7.00</td>
<td>0.00</td>
<td>-0.07</td>
</tr>
</tbody>
</table>

Table 3.12 Measured volume by gas dilution versus syringe volume for the Innocor SF₆ analyser
Figure 3-17 Measured volume by gas dilution versus syringe volume for the Innocor N$_2$O analyser.

The dashed line is the line of identity

<table>
<thead>
<tr>
<th>Syringe volume (L)</th>
<th>Measured volume (L)</th>
<th>Error (L)</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2.05</td>
<td>0.05</td>
<td>2.54</td>
</tr>
<tr>
<td>3</td>
<td>3.05</td>
<td>0.05</td>
<td>1.77</td>
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<tr>
<td>4</td>
<td>3.96</td>
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<td>4.92</td>
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<tr>
<td>6</td>
<td>5.96</td>
<td>-0.04</td>
<td>-0.72</td>
</tr>
<tr>
<td>7</td>
<td>6.99</td>
<td>-0.01</td>
<td>-0.15</td>
</tr>
</tbody>
</table>

Table 3-13 Measured volume by gas dilution versus syringe volume for the Innocor N$_2$O analyser
3.3. Computed tomography

3.3.1. Scoring of CT parenchymal abnormalities

Patients were scanned on a 64-channel multidetector CT machine (GE 64 VCT Lightspeed machine; GE Healthcare, Waukesha, Wisconsin, USA; 100 kV, Auto mA/Smart mA; pitch 1.375, 0.5 sec tube rotation, beam collimation 40 mm, detector size 0.65 mm). Contrast was injected at a rate of 4-5 ml/s and scanning was performed cranio-caudally with the subject in a supine position and breathing suspended at maximal inspiration. The period of CT scanning was timed to coincide with optimal contrast opacification of the pulmonary arterial tree using bolus-tracking. Interspaced HRCT images (1.5mm collimation at 10 mm intervals) had been acquired at total lung capacity, in the supine and prone positions. Images were reconstructed using a high-spatial-frequency (bone) algorithm. All CT studies were securely stored on CD for subsequent review.

The following CT patterns [176] were quantified to the nearest 5%:

i) A reticular pattern defined as innumerable interlacing line shadows which, by summation, produce an appearance resembling a mesh.

ii) Ground-glass opacification (defined as a hazy increased lung opacity in which the visibility of bronchial and vascular margins was preserved).
iii) Areas of decreased attenuation as part of a mosaic attenuation pattern defined as a patchwork of areas of differing lung densities.

iv) Consolidation, defined as a homogeneous increase in lung parenchymal attenuation which obscures the margins of airway and vessel walls. In patients with consolidation, the extent to the nearest 5% of sub-pleural consolidation was also recorded.

For ground glass opacification, a score was assigned based on the observed extent (0=none; 1= <10% extent; 2= 10-50% extent; 3 >50% extent).

The following CT signs were scored semi-quantitatively:

i) Thickening of interlobular septa (0=none; 1= <5 thickened interlobular septa [ILS]; 2= >5 thickened ILS or 4 <50% pleural surface involved; 3= >50% pleural surface involved or 4 = diffusely thickened ILS).

ii) Lobar volume loss (0=none; 1=mild or 2=severe).

The presence or absence of the following signs was recorded:

i) Traction bronchiectasis defined as irregular bronchial/bronchiolar dilatation caused by surrounding retractile pulmonary fibrosis in a reticular pattern and/or ground-glass opacification
ii) Linear bands of attenuation.

iii) Sub-pleural curvilinear lines defined as a thin curvilinear opacity of 1-3 mm thickness and lying within one centimetre of and parallel to the pleural surface.

iv) Pulmonary infarcts defined as a peripheral irregular opacity associated with a linear opacity no more than 2 cm in length.

Whole-lung scores were produced by taking the mean of all lobes for signs which had been measured to the nearest 5% and by summing the lobar scores for signs recorded as present/absent.

3.3.2. Measurement of CT vascular dimensions

Vascular dimensions were assessed in two ways. First, proprietary electronic callipers were used to measure the widest short axis diameters of the upper lobe apical or apico-posterior segmental arteries and the lower lobe posterobasal segmental arteries together with the widest external short axis diameter of the corresponding segmental bronchi on the same axial image in at least three out of four lobes [177]. Based on those measurements, the mean segmental artery/bronchus (A/B) ratio in at least three out of four lobes was calculated.
Distal vessel dimensions, including both arteries and veins at the subsubsegmental level [178] were measured using the method of Matsuoka et al [179, 180]. Three CT slices were selected from each examination. The upper cranial slice was located approximately one centimetre above the upper margin of the aortic arch, the middle slice approximately one centimetre below the carina, and the lower caudal slice approximately one centimetre below the right inferior pulmonary vein. The measurements were performed using the Java-based semi-automated image analysis software ‘ImageJ’ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2012). Before processing, each selected image was smoothed using Gaussian blurring to filter image noise. The lung field was then segmented using a thresholding technique to include all pixels between -500 and -1024 Hounsfield Units (HU) (Figure 3.18A) and the segmented image was converted to a binary image with a window level of -760 HU (Figure 3.18B). The ‘Analyze Particles’ function was then used to count and measure the cross-sectional area of vessels within a range of 0-5mm². The ‘Circularity’ function was then used to select only those vessels running approximately perpendicular to the scan plane based on their apparent shape in the slice image, setting the circularity range to 0.9-1.0 (Figure 3.18C). The total summated cross-sectional area for those vessels was then expressed as a percentage of the total lung area of the three selected slices using threshold values between -500 HU and -1024 HU (CSA<5mm%).
Figure 3-18 Measurement of small vessel cross-sectional area (CSA)<5 mm% using ImageJ software.

(A) High-resolution CT image of lung field segmented with threshold values of −500 to −1024 HU. (B) Segmented image converted to binary image with window level of −720 HU, with pulmonary vessels displayed in black. (C) Mask image for particle analysis with vessel size set to 0–5mm2 and the circularity from 0.9 to 1.0.
The segmental A/B ratio has been shown to correlate with pulmonary artery pressure in a mixed cohort of patients with lung disease [181]. CSA<5mm% has been measured in patients with HRCT-defined emphysema due to COPD and has been shown to correlate with pulmonary artery pressure in this group [179, 180].

CT total lung volume (TLV\textsubscript{CT}), comprising air plus tissue components, was derived using a proprietary lung segmentation algorithm (Apollo, Vida Core Lab Services, Vida Diagnostics Inc, Iowa, USA) which uses a localized and adaptive threshold method to delineate lung tissue on a lobar basis from surrounding tissues (Figure 3.19). The TLV\textsubscript{CT} was not available for the scans obtained in 2003-2005.
For a subset of patients in whom longitudinal measurements are available, initial and follow-up CT scans were compared. Two observers identified anatomically comparable sections as judged by vascular and bronchial landmarks on the two CT scans from each individual and recorded their impression of whether the overall appearance of the interstitium on the follow-up scan compared to the initial scan had deteriorated. Patients who had deteriorated were assigned a ‘gestalt’ change score of ‘one’ and those who did not were scored as zero.
3.4. Echocardiography

Two and three dimensional echocardiography was performed using digital acquisitions on a Phillips Sonos 7500 ultrasound system and a 2.5 MHz matrix array with standalone transducers (Philips Medical Systems BV, The Netherlands). Scans were performed according to American Society of Echocardiography guidelines [182] by a single expert cardiographer. Images over multiple views were obtained over at least three cardiac cycles; tricuspid and mitral inflow recordings were obtained using pulsed Doppler. Tricuspid regurgitant jet velocity (TRV) was measured by continuous-wave Doppler: the largest value measured in any of four standard views was reported.

Valvular regurgitation was graded from Doppler determinations of trans-valvar flow. Tricuspid regurgitation (TR) was assessed in the parasternal right ventricular inflow, parasternal short axis and apical four-chamber views. A minimum of five sequential complexes were recorded. Continuous-wave Doppler signals of the peak regurgitant jet velocities (TRV) (normal <2.5 m/s) were used to estimate the right ventricular systolic pressures (RVSP) using the Bernoulli equation:

\[ RVSP = 4 \cdot TRV^2 \]

PAP (normal <25 mm Hg) was calculated as the sum of RVSP and right atrial systolic pressures. Cardiac output (CO) (normal range 4.0–8.0 L/s) and right-ventricular
diastolic volumes (RVDV) (normal range 100–160 mL) were also measured. Right and left ventricular function were assessed by measurement of the tricuspid annular plane systolic excursion (TAPSE) (normal >1.5 cm) and the ratio of early diastolic LV inflow (E) to lateral mitral annulus velocity (e’) measured by tissue Doppler (E/e’) (normal <8).
3.5. **Statistical methods**

Comparisons between groups were performed using Mann-Whitney U or Wilcoxon signed-rank tests for unpaired and paired data respectively. The strength of relationships between variables was assessed using Spearman’s rank correlation. Tests of proportions between groups were performed using Fisher’s exact test. Adjustments for multiplicity were not performed, since the majority of individual statistical tests conducted within each study were complementary (i.e. not independent). This is the case for tests on the subdivisions of lung volume and forced expiratory flows (which are dependent on lung volume) and for the correlated multiple-flow exhaled nitric oxide measurements in Chapter Seven.

Correction of p-values for multiple testing (e.g.: by Bonferroni adjustment) is generally only appropriate where individual statistical tests are independent. Nevertheless, there remains the possibility of an increased rate of type two errors due to the large number of statistical tests conducted – it is recommended, therefore, that the results from each study are considered in their totality, particularly in terms of internal consistency, rather than regarding each significant p-value as a definitive test for an individual null hypothesis.

Multiple linear regression was used to test for associations between lung function and morphologic and haematological variables. Regression diagnostics were performed to ensure that all models satisfied the assumptions of multiple linear regression as determined by assessment of homoscedasticity and the absence of multicollinearity between predictors. Where the condition of homoscedasticity was not satisfied, analyses were performed on the log-transformed dependent
variable to normalise the residuals. For binary outcome variables, logistic regression was used. Linear mixed-effect models (LMMs) were used to analyse longitudinal data. Statistical testing was performed using Prism (Graphpad Software Inc., San Diego, CA), SPSS (IBM Corp. Armonk, NY) or R (version 3.1.1, R Foundation for Statistical Computing, Vienna, Austria) software. Power analysis was performed using G*Power software (3.1.3 Heinrich Heine Universität, Düsseldorf, Germany) [183, 184].
4. PULMONARY FUNCTION, CT AND ECHOCARDIOGRAPHIC ABNORMALITIES IN SICKLE CELL DISEASE

Note: All lung function tests and statistical analyses were performed by the candidate. Parenchymal abnormalities on HRCT were scored by Dr Sujal Desai (King’s College Hospital NHS Foundation Trust) and Prof David Hansell (Royal Brompton & Harefield NHS Foundation Trust). Echocardiography was performed by Dr Sitali Mushemi (King’s College London) Prof Atholl Wells (Royal Brompton & Harefield NHS Foundation Trust) provided statistical support.

4.1 Introduction

Adults with SCD can suffer from parenchymal lung disease and pulmonary vascular disease or both; affected individuals can suffer premature death. A previous study conducted by this research group demonstrated that the majority of a cohort of adult patients with SCD had pulmonary abnormalities on high-resolution CT (HRCT) [73]. The HRCT findings significantly correlated with pulmonary function testing results; in particular, there were correlations between reductions in FVC and FEV1 and the prominence of the central vessels on HRCT. Prominent central vessels were found on HRCT in eight of the nine patients with restrictive abnormalities. The prominent central vessels may reflect the raised pulmonary capillary blood volume as a result of chronic anaemia causing an increased cardiac output (CO) [123] and dilation of the pulmonary vessels [185].
This study was conducted, therefore, to test the hypothesis that enlarged pulmonary vessels would be associated with elevated cardiac output, impaired ventricular function, and lung function abnormalities. This was achieved by prospectively undertaking HRCT studies, lung function tests and echocardiographic assessments. A further aim of this study was to reassess the cohort examined seven years ago [73] to determine whether any decline in lung function correlated with vascular changes evidenced by HRCT studies.

4.2 Methods

Adults with SCD were assessed between 2009 and 2013. Subjects included in the study of Sylvester et al had also initially been assessed between 2003 and 2005. The study was approved by King’s College Hospital Research Ethics Committee (LREC 02-0080). All participants gave written informed consent.

4.2.1 Lung function assessments

FEV$_1$, vital capacity (VC), forced expiratory flow between 25 and 75% of VC (FEF$_{25-75}$), total lung capacity (TLC), residual volume (RV) and mean respiratory system resistance (Rrs) were measured as previously described (Section 3). Results were expressed as percent predicted for height, age, and sex using the ethnic-specific reference equations for spirometry [186] and the European Community for Steel
and Coal Statement of the European Respiratory Society reference equations for lung volumes and gas transfer [187].

The mean respiratory system resistance ($R_{rs}(0)$) and frequency dependence of resistance ($R_{rs}(1)$) from 5-25Hz were measured using impulse oscillometry and expressed as the percent predicted for height, weight, and age [188]. The frequency dependence of resistance was assessed using the mean slope of the resistance-frequency curve over the range 5 to 25Hz. The $R_{rs}(0)$ and $R_{rs}(1)$ were used as the only published predicted values for $R_{rs}$ in adults report these indices but do not report $R_{rs}$ at individual oscillation frequencies and normalisation to predicted value is necessary to account for known age and sex related variation in $R_{rs}$ [189]. Respiratory resistance was considered to be elevated if $R_{rs}(0)$ was greater than the upper limit of normal, defined as a standard-deviation score of greater than 1.64, based on the reference data of Pasker et. al [188]. Spirometry was repeated following administration of a bronchodilator (400μg salbutamol via a MDI and spacer: the MDI was vigorously shaken between puffs and the delivery of each puff was followed by five normal breaths) and a positive response was defined as an increase in FEV1 of greater than or equal to 12% from baseline and an increase of at least 200ml. Oxygen saturation was measured using a pulse oximetry (Masimo Radical 7 and a rainbow probe, Masimo, California, USA).

The predicted values for total lung capacity were reduced by 12%, and residual volume by 7% to correct for ethnicity [136]. The lower and upper limits of normal were defined as the fifth and ninety-fifth percentiles respectively of the appropriate reference range. Patients were diagnosed as having a restrictive
abnormality if their TLC was less than the lower limit of normal (LLN), with a normal FEV1:VC. An obstructive abnormality was diagnosed if the FEV1:VC was less than the LLN with a normal VC, or if VC was less than the LLN with a normal TLC and an RV:TLC greater than the upper limit of normal (ULN). A mixed abnormality was diagnosed if the TLC and FEV1:VC were less than the LLNs.

4.2.2 Computed Tomography

CT patterns were quantified independently by two thoracic radiologists (SD and DH) blinded to the clinical and functional data: for the longitudinal analysis, scans at the two time points were assessed blinded. The extent and severity of CT abnormalities were recorded in individual lobes, the lingula being considered a separate lobe for scoring purposes. Distal vessel dimensions, including both arteries and veins at the sub-subsegmental level were measured using the method of Matsuoka et al. as previously described. The total summated cross-sectional area for the vessels was then expressed as a percentage of the total lung area of the three selected slices using threshold values between −500 and −1024 HU (cross-sectional area (CSA)<5 mm%). The mean segmental artery/bronchus (A/B) ratio in at least three of four lobes was calculated. CT total lung volume (TLV_{CT}) was derived using a proprietary lung segmentation algorithm. For the longitudinal analyses, initial and follow-up CT scans were compared. Two observers identified anatomically comparable sections as judged by vascular and bronchial landmarks on the two CT scans from each individual and recorded their impression of whether
the overall appearance of the interstitium on the follow-up scan compared to the initial scan had deteriorated. Patients who had deteriorated were assigned a ‘gestalt’ change score of ‘one’ and those who did not were scored as ‘zero’.

4.2.3 Echocardiography

Two-dimensional and three-dimensional transthoracic echocardiography was performed according to the international guidelines as previously described (section 3). TRV was used to derive RVSP. Cardiac output (CO) (normal range 4.0–8.0 L/s) and right-ventricular diastolic volumes (RVDV) (normal range 100–160 mL) were also measured. Right and left ventricular function were assessed by measurement of the tricuspid annular plane systolic excursion (TAPSE) (normal >1.5 cm) and the ratio of early diastolic LV inflow (E) to lateral mitral annulus velocity (e’) measured by tissue Doppler (E/e’) (normal <8). Technically acceptable TAPSE measurements were available in 32 patients and E/e’ in 33 patients.

4.2.4 Haematological and clinical data

Haemoglobin, lactate dehydrogenase (LDH), bilirubin levels and reticulocyte counts were obtained from routine blood tests taken within one month of testing when patients were clinically stable. A review of medical records was conducted to determine historical ACS status. Complete records were obtained for the previous twenty-eight to thirty-six years in thirty out of thirty-five patients (86%); for the
remaining three patients, records were available only for the nine to twelve years prior to the study visit. Patients were deemed to have a history of ACS if they had suffered at least one episode of chest pain, dyspnoea and pyrexia together with a new pulmonary infiltrate on chest radiograph [78].

4.2.5 Statistical analysis

Data were tested for normality using the D’Agostino and Pearson omnibus normality test. Since data were not normally-distributed, comparisons were made with Mann-Whitney U tests and paired analyses were performed using paired-sample Wilcoxon matched-pairs signed rank tests. In the longitudinal cohort, non-parametric tests were used for baseline-follow up comparisons as the number of patients was too small too meaningfully perform normality testing. For the descriptive data in the larger cohort (n=35), who were tested once, data were displayed as median (range) to demonstrate the wide range in lung function. On HRCT, whilst GGO score, bands, curvilinear bands, and A/B ratio were not normally distributed, the residuals in all of the final regression models did not differ significantly from a normal distribution (Pearson omnibus normality test p>0.05). The strength of relationships were assessed using the Spearman rank correlation. Stepwise linear regression with backward elimination was used to identify HRCT parenchymal and vascular results which correlated with the results of the lung function tests. HRCT variables examined in the preceding bivariate analyses were entered as initial predictors, unless predictors were multi-collinear. All regression
models were built in the sample size n=35. All final models satisfied the assumptions of multiple linear regression. In the longitudinal cohort, exploratory models were generated to test whether baseline vascular markers predicted subsequent deterioration of parenchymal disease and progression of lung function abnormalities. Logistic regression was used to assess the effect of baseline segmental A/B ratio and CSA<5mm on the deterioration in the overall CT appearance (‘Gestalt’ change score) and linear mixed model analysis (LMM) to predict individual decline in lung function results.

4.3 Results

4.3.1 Subjects

Thirty-five patients with a median age of 43 (range 17-73) years were assessed. Twenty of the 35 patients (median age at initial assessment 38, range 17 – 66 years) had been assessed at a median of 6.6 (range 5.5-6.7) years previously. Of the thirty-three patients who participated in the original study 20 were tested at follow-up: three had died, six were lost to follow-up three declined to participate in the follow-up study and one declined to undergo HRCT scanning and was excluded. The twenty patients who were tested at follow-up were included in the 2009-2013 cohort, together with fifteen new patients making a total of thirty-five patients (Figure 4.1). Patient characteristics for the combined cohort of thirty-five patients in the new analysis are given in table 4.1.
Table 4-1 Clinical characteristics of the entire cohort (n=35).

Results are given as median (range) except where indicated*
4.3.2 Lung function test results

There was a wide variation in the lung function of the cohort (table 4.2). In total, 28 of the 35 patients (80%) had lung function abnormalities: 8 (23%) had a restrictive abnormality, 6 (17%) an obstructive abnormality, 4 (11%) a mixed abnormality and 10 (29%) an isolated reduction in carbon monoxide diffusing capacity (DLCO). Six patients (17%) had a significant response to bronchodilator; three had an obstructive, one a mixed and one a restrictive abnormality, and one no lung function abnormality. Sixteen patients (46%) had elevated $R_{ns}$; six of whom otherwise had normal lung function. Figure 4.2 shows the SD-score for TLC and FEV$_1$:VC.
<table>
<thead>
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<td>FEV&lt;sub&gt;1&lt;/sub&gt;</td>
<td>77.4 (27.4–114.2)</td>
</tr>
<tr>
<td>VC</td>
<td>81.3 (27.8–113.0)</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;:VC</td>
<td>96.3 (75.9–115.8)</td>
</tr>
<tr>
<td>FEF&lt;sub&gt;25-75&lt;/sub&gt;</td>
<td>69.6 (13.7–129.2)</td>
</tr>
<tr>
<td>TLC</td>
<td>87.8 (63.8–109.6)</td>
</tr>
<tr>
<td>RV</td>
<td>93.5 (50.1–162.6)</td>
</tr>
<tr>
<td>RV:TLC</td>
<td>106.3 (64.6–197.4)</td>
</tr>
<tr>
<td>DLCOc</td>
<td>73.6 (43.9–104.8)</td>
</tr>
<tr>
<td>KCOc</td>
<td>94.0 (67.5–124.8)</td>
</tr>
<tr>
<td>Rrs(0)</td>
<td>128.2 (79.4–209.0)</td>
</tr>
<tr>
<td><strong>Rrs(1) (kPa L&lt;sup&gt;−1&lt;/sup&gt; s&lt;sup&gt;−1&lt;/sup&gt; Hz)</strong></td>
<td>0.018 (0.001–0.042)</td>
</tr>
<tr>
<td><strong>SpO&lt;sub&gt;2&lt;/sub&gt; (%)</strong></td>
<td>96.0 (85.0–100.0)</td>
</tr>
</tbody>
</table>

Table 4-2 Lung function test results in the whole cohort for the CT study (n=35).

Includes the follow-up visit for the subgroup with longitudinal measurements.

*Data are presented as per cent predicted for height, age and sex except where indicated
Figure 4-2 TLC and FEV₁:VC expressed as z-scores for the whole cohort, showing the presence of restriction and airflow obstruction.

The dashed line indicates the cut-off for an abnormally low result.
4.3.3 HRCT abnormalities

A reticular pattern on HRCT was the most prevalent and extensive abnormality, seen in 26 patients (median 5 (range 0–17.5) %). Ground glass opacification was present in nine patients (median extent 0 (range 0–3)). Consolidation was seen in nine patients (median extent 0.4 (0–4.2)). Reduction in lobar volume was seen in 19 patients (median severity 1 (range 0–7)). Linear bands were seen in 23 patients (median 1 (range 0–6)) and subpleural curvilinear lines in 9 patients (median 0 (range 0–3)). Subpleural consolidation (n=6), thickened interlobular septa (n=2), infarcts (n=7) and traction bronchiectasis (n=0) were present in a minority of patients and excluded from further analysis. The median A/B ratio was 1.30 (range 1.0–2.50) and CSA<5 mm% 0.48 (range 0.22–10.2). The extent of a reticular pattern was positively correlated with the presence of linear bands (r=0.64, p<0.0001) and ground glass opacification score (r=0.42, p=0.0115). The presence of linear bands was positively correlated with subpleural curvilinear lines (r=0.39, p=0.0204), which were positively related to the A/B ratio (r=0.46, p=0.0056). The A/B ratio and CSA<5 mm% were positively correlated (r=0.72, p<0.0001).

4.3.4 Relationships between pulmonary function and HRCT results

Subpleural curvilinear lines were negatively correlated with FEV1 (r=−0.39, p=0.0230) and FEF25−75 (r=−0.44, p=0.0403), and the extent of a reticular pattern was negatively correlated with Rrs(0) (r=−0.40, p=0.0198). TLV<sub>CT</sub> was positively
correlated with FEV1 (r=0.57, p=0.0014), VC (r=0.66, p < 0.0001) and TLC (r=0.82, p<0.0001). The segmental A/B ratio was negatively correlated with FEV1 (r=−0.53, p=0.0011), VC (r=−0.48, p=0.0036), FEF25-75 (r=−0.44, p=0.0403) and SpO2 (r=−0.47, 0.0037), and positively with RV:TLC (r=0.45, p=0.0073), and Rrs(1) (r=0.22, p=0.0267) (Figure 4.3). The CSA<5 mm% was negatively correlated with FEV1 (r=−0.71, p<0.0001), VC (r=−0.72, p<0.0001), FEF25-75 (r=−0.51, p=0.0010) and SpO2 (r=−0.44, p=0.0070), and positively to RV (r=0.51, p=0.0020), RV:TLC (r=0.695, p<0.0001), Rrs(0) (r=0.35, p=0.0417) and Rrs(1) (r=0.67, p<0.0001) (Figure 4.4).

The segmental A/B ratio and CSA<5 mm% exhibited a strong degree of multicollinearity. Therefore, separate models were generated with segmental A/B ratio or CSA<5 mm% as predictors. On stepwise regression, the segmental A/B ratio was independently related to a reduced FEV1, VC, FEF25/75 and SpO2 and to an increased RV, RV:TLC, Rrs(0) and Rrs(1) (table 4.3). Linear bands were independently linked to a reduced FVC and TLC (table 4.3).The CSA<5 mm% was independently linked to a reduced FEV1, VC, FEF25-75 and SpO2 and to an increased RV, RV:TLC, Rrs(0) and Rrs(1) (table 4.4). The extent of a reticular pattern was associated with a reduced Rrs(0) and Rrs(1) (table 4.4). On multiple linear regression, restrictive and mixed obstructive/restrictive, but not purely obstructive defects, were associated with a significant reduction in TLV_{CT} (β= -1.092, p=0.0045; β= -1.231, p=-0.0212; β = -0.014, p=0.9783), suggesting that CT measurement of lung volume may provide an alternative method of diagnosing restrictive lung disease in patients unable to perform lung function tests.
<table>
<thead>
<tr>
<th>Lung function</th>
<th>HRCT</th>
<th>R²</th>
<th>β (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV₁</td>
<td>Segmental A/B ratio</td>
<td>0.40</td>
<td>-0.636 (-0.937 to -0.335)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>VC</td>
<td>Segmental A/B ratio</td>
<td>0.42</td>
<td>-0.561 (-0.855 to -0.267)</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>Bands</td>
<td></td>
<td>-0.288 (-0.563 to -0.013)</td>
<td>0.0397</td>
</tr>
<tr>
<td>FEF₂₅₋₇₅</td>
<td>Segmental A/B ratio</td>
<td>0.19</td>
<td>-0.447 (-0.772 to -0.122)</td>
<td>0.0070</td>
</tr>
<tr>
<td>TLC</td>
<td>Bands</td>
<td>0.11</td>
<td>-0.336 (-0.670 to -0.001)</td>
<td>0.0484</td>
</tr>
<tr>
<td>RV</td>
<td>Segmental A/B ratio</td>
<td>0.32</td>
<td>0.478 (0.168 to 0.788)</td>
<td>0.0026</td>
</tr>
<tr>
<td></td>
<td>GGO score</td>
<td></td>
<td>-0.344 (-0.645 to -0.043)</td>
<td>0.0249</td>
</tr>
<tr>
<td>RV:TLC</td>
<td>Segmental A/B ratio</td>
<td>0.40</td>
<td>0.623 (0.312 to 0.934)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Rₛ(0)</td>
<td>Segmental A/B ratio</td>
<td>0.32</td>
<td>0.431 (0.104 to 0.758)</td>
<td>0.0098</td>
</tr>
<tr>
<td></td>
<td>Reticular pattern</td>
<td></td>
<td>-0.497 (-0.827 to -0.167)</td>
<td>0.0032</td>
</tr>
<tr>
<td>Rₛ(1)</td>
<td>Segmental A/B ratio</td>
<td>0.33</td>
<td>0.554 (0.232 to 0.876)</td>
<td>0.0008</td>
</tr>
<tr>
<td></td>
<td>Reticular pattern</td>
<td></td>
<td>-0.316 (-0.620 to -0.012)</td>
<td>0.0414</td>
</tr>
<tr>
<td>SpO₂</td>
<td>Segmental A/B ratio</td>
<td>0.22</td>
<td>-0.466 (-0.789 to -0.143)</td>
<td>0.0047</td>
</tr>
</tbody>
</table>

Table 4-3 Results of multivariate analysis with the segmental A/B ratio as a predictor.

HRCT, high-resolution CT; GGO, ground glass opacification; RV:TLC, residual volume:total lung capacity ratio; VC, vital capacity.
Figure 4-3 Relationship between lung function tests and segmental artery-bronchus ratio on HRCT.

Lung function tests are expressed as percentage of predicted except where indicated. Lines represent the least-square linear regression best fit in each group, the shaded areas are the 95% confidence interval for the fit.
<table>
<thead>
<tr>
<th>Lung function</th>
<th>HRCT</th>
<th>$R^2$</th>
<th>$\beta$(95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV$_1$</td>
<td>CSA&lt;5 mm%</td>
<td>0.52</td>
<td>$-0.666 (-0.948$ to $-0.384)$</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Curvilinear bands</td>
<td></td>
<td>$-0.213 (-0.458$ to $0.032)$</td>
<td>0.0885</td>
</tr>
<tr>
<td>VC</td>
<td>CSA&lt;5 mm%</td>
<td>0.51</td>
<td>$-0.721 (-1.006$ to $-0.436)$</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FEF$_{25-75}$</td>
<td>CSA&lt;5 mm%</td>
<td>0.26</td>
<td>$-0.508 (-0.826$ to $-0.190)$</td>
<td>0.0018</td>
</tr>
<tr>
<td>TLC</td>
<td>Bands</td>
<td>0.11</td>
<td>$-0.336 (-0.670$ to $-0.002)$</td>
<td>0.0484</td>
</tr>
<tr>
<td>RV</td>
<td>CSA&lt;5 mm%</td>
<td>0.36</td>
<td>0.518 (0.213 to 0.823)</td>
<td>0.0009</td>
</tr>
<tr>
<td>RV:TLC</td>
<td>CSA&lt;5 mm%</td>
<td>0.61</td>
<td>0.763 (0.468 to 1.058)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$R_{rs}(0)$</td>
<td>CSA&lt;5 mm%</td>
<td>0.34</td>
<td>0.422 (0.122 to 0.722)</td>
<td>0.0058</td>
</tr>
<tr>
<td></td>
<td>Reticular pattern</td>
<td></td>
<td>$-0.516 (-0.836$ to $-0.196)$</td>
<td>0.0016</td>
</tr>
<tr>
<td>$R_{rs}(1)$</td>
<td>CSA&lt;5 mm%</td>
<td>0.58</td>
<td>0.748 (0.458 to 1.038)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Reticular pattern</td>
<td></td>
<td>$-0.516 (-0.964$ to $-0.068)$</td>
<td>0.0237</td>
</tr>
<tr>
<td>SpO$_2$</td>
<td>CSA&lt;5 mm%</td>
<td>0.36</td>
<td>$-0.593 (-0.913$ to $-0.273)$</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

Table 4-4 Results of the multivariate analysis with CSA <5 mm% as a predictor.

HRCT, high-resolution CT; RV:TLC, residual volume:total lung capacity ratio; VC, vital capacity.
Figure 4-4 Relationship between lung function tests and CSA<5mm% on HRCT.

Lung function tests are expressed as percentage of predicted except where indicated. Lines represent the least-square linear regression best fit in each group, the shaded areas are the 95% confidence interval for the fit.
4.3.5 Echocardiography results

The median TRV was 2.65 (range 1.19–3.60) (m/s), the median estimated PASP 33.5 (range 9.0–70.0) (mm Hg), the median CO 6.0 (range 3.3–9.1) (L/min) and the median RVDV 93.9 (range 36.7–182.6) (mL). The median TAPSE was 2.30 (1.00–3.95) (cm), and the median E/e’ ratio was 7.60 (4.70–16.60). Significant correlations were observed between CO and the A/B ratio (r=0.41, p=0.0120) and the CSA<5 mm% (r=0.35, p=0.0440), but not with the estimated PAP. There was a correlation of CSA<5 mm% with E/e’ (r=0.45, p=0.009). There was also a correlation of CSA<5 mm% with TAPSE (r=−0.38, p=0.035), but only one subject had an abnormal TAPSE result (<1.5) and thus it is not possible to comment on the relationship between HRCT indices of vascular dilatation and echo indices of RV dysfunction.

The segmental A/B ratio and the CSA<5 mm% were significantly correlated with the haemoglobin level (r=−0.50, p=0.0021; r=−0.42, p=0.0090, respectively) and the lactate dehydrogenase (LDH) level (r=0.33, p=0.0450; r=0.33, p=0.0492, respectively). The segmental A/B ratio was related to the bilirubin level (r=0.47, p=0.0037) and the CSA<5 mm% to the reticulocyte count (r=0.39, p=0.0167).
4.3.6 Longitudinal analysis

The lung function results of the 20 patients who were reassessed had declined significantly (table 4.5, Figure 4.5). There were, however, no significant changes seen in the prevalence or extent/severity of lung parenchymal abnormalities on CT, but both the median segmental A/B ratio and CSA<5 mm% had increased significantly over the follow-up period (table 4.6). Four patients (20%) had a gestalt change score of one. The percentage change from baseline in the A/B ratio correlated negatively with that in FEV1 (r=0.330, p=0.0156) and carbon monoxide transfer coefficient, corrected for haemoglobin concentration (KCOc) (r=−0.554, p=0.0139) and positively with that in RV (r=0.475, p=0.0342) and RV:TLC (r=−0.557, p=0.0107). The percentage change from baseline in CSA<5 mm% correlated negatively with that in FEV1 (r=−0.330, p=0.0079) and VC (r=−0.487, p=0.0357) and positively with RV:TLC (r=0.557, p=0.0164). Logistic regression demonstrated that a higher CSA<5 mm% at baseline was predictive of a subsequent overall deterioration in HRCT appearance, that is, a gestalt change score of 1 (OR 2.62 (95% CI 1.14 to 6.01) per unit increase in CSA<5 mm%, p=0.023). Linear mixed model analysis demonstrated a significant association of baseline CSA<5 mm% with subsequent decline in TLC, with an increased decline of −1.84% (95% CI −3.31 to −0.37) per year for each unit increase in CSA<5 mm% at baseline, p=0.014. CSA<5 mm% was also predictive of a more rapid change in RV:TLC, with a change in slope of +4.93% (95% CI 0.88 to 8.99) per year per unit increase in CSA<5 mm% at baseline, p=0.017. Plots of the percentage change from baseline in TLC and RV:TLC versus CSA<5mm% at baseline are given in Figure 4.6.
Table 4-5 Pulmonary function results at initial and follow-up assessment in the subgroup with longitudinal measurements (n=20).

Data are expressed as median (range) and presented as per cent predicted for height, age and sex unless indicated*. RV:TLC, residual volume:total lung capacity ratio; VC, vital capacity. † Wilcoxon signed-rank test.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=20</td>
<td>n=20</td>
<td></td>
</tr>
<tr>
<td>FEV₁</td>
<td>80.6 (41.9–115.4)</td>
<td>70.9 (25.5–98.4)</td>
<td>0.0004</td>
</tr>
<tr>
<td>VC</td>
<td>80.5 (48.4–102.3)</td>
<td>74.5 (27.1–106.6)</td>
<td>0.0347</td>
</tr>
<tr>
<td>FEV₁:VC</td>
<td>104.8 (90.9–129.3)</td>
<td>98.7 (74.0–113.0)</td>
<td>0.0261</td>
</tr>
<tr>
<td>FEF₂⁵⁻⁷⁵</td>
<td>87.0 (33.5–136.3)</td>
<td>70.1 (19.6–129.2)</td>
<td>0.0033</td>
</tr>
<tr>
<td>TLC</td>
<td>87.6 (67.2–107.0)</td>
<td>85.5 (63.8–109.6)</td>
<td>0.5628</td>
</tr>
<tr>
<td>RV</td>
<td>87.6 (46.3–125.0)</td>
<td>90.0 (50.1–162.6)</td>
<td>0.1213</td>
</tr>
<tr>
<td>RV:TLC</td>
<td>102.0 (56.1–160.4)</td>
<td>111.1 (64.6–197.0)</td>
<td>0.0383</td>
</tr>
<tr>
<td>DLCOc</td>
<td>74.1 (42.7–104.1)</td>
<td>74.3 (45.3–104.8)</td>
<td>0.9879</td>
</tr>
<tr>
<td>KCOc</td>
<td>111.5 (85.0–154.9)</td>
<td>104.8 (77.0–137.8)</td>
<td>0.0325</td>
</tr>
<tr>
<td>SpO₂(%)*</td>
<td>97 (84–99)</td>
<td>97 (85–100)</td>
<td>0.4380</td>
</tr>
</tbody>
</table>
Figure 4-5 Pulmonary function results at initial and follow-up assessment.

Lung function tests are expressed as percentage of predicted except where indicated. Individual data are shown by linked data points.
<table>
<thead>
<tr>
<th>HRCT pattern</th>
<th>Initial</th>
<th>Follow-up</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extent/severity</td>
<td>Extent/severity</td>
<td>Value†</td>
</tr>
<tr>
<td>Reticular pattern</td>
<td>5.4 (0.0–17.5)</td>
<td>5.8 (0–17.5)</td>
<td>0.1987</td>
</tr>
<tr>
<td>Ground glass</td>
<td>0 (0–3)</td>
<td>0 (0–3)</td>
<td>0.3458</td>
</tr>
<tr>
<td>opacification score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decreased attenuation</td>
<td>1.3 (0.0–16.7)</td>
<td>1.3 (0.0–16.7)</td>
<td>0.9793</td>
</tr>
<tr>
<td>Consolidation</td>
<td>0.0 (0.0–4.2)</td>
<td>0.0 (0.0–1.7)</td>
<td>0.5000</td>
</tr>
<tr>
<td>Lobar volume loss</td>
<td>1 (0–7)</td>
<td>1 (0–7)</td>
<td>1.0000</td>
</tr>
<tr>
<td>Linear bands</td>
<td>1 (0–5)</td>
<td>1.5 (0–6)</td>
<td>0.0961</td>
</tr>
<tr>
<td>Subpleural curvilinear lines</td>
<td>0 (0–2)</td>
<td>0 (0–2)</td>
<td>0.0890</td>
</tr>
<tr>
<td>A/B ratio</td>
<td>1.26 (0.90–1.70)</td>
<td>1.50 (1.05–2.50)</td>
<td>0.0347</td>
</tr>
<tr>
<td>CSA&lt;5 mm%</td>
<td>0.26 (0.14–0.52)</td>
<td>0.54 (0.22–1.10)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 4-6  High-resolution CT (HRCT) parenchymal and vascular analysis at initial and follow-up assessment.

Data are presented as median (range). † Wilcoxon signed-rank test.
Figure 4-6  Percentage change from baseline for TLC and RV:TLC versus baseline CSA<5mm% in the subgroup (n=20) with longitudinal measurements.
4.4 Discussion

These data demonstrate that pulmonary vascular abnormalities on HRCT are significantly related to pulmonary function impairment in adults with SCD. The segmental A/B ratio and CSA<5 mm% were independently linked to reductions in FEV$_1$, VC and FEF$_{25/75}$ and to increased respiratory system resistance and RV:TLC. In addition, small vessel size correlated with reduced oxygen saturation and haemoglobin concentration and increased LDH, bilirubin and reticulocyte levels. Those results suggest relationships between anaemia, haemolysis, hypoxia and pulmonary function abnormalities. A positive correlation was observed between CSA<5 mm% (a measure of distal arteries and veins) and E/e’ which is a marker of left atrial filling pressure that is elevated if there is LV diastolic dysfunction. A higher E/e’ would result in some elevation of pulmonary venous pressure, and this result suggests a role for precapillary and postcapillary pulmonary vascular changes in SCD-related lung disease. There was a negative relationship with vascular dimensions and VC, FEV$_1$, FEF$_{25-75}$ and SpO2 and a positive relationship with RV, RV:TLC, $R_s$(0) and $R_s$(1), suggesting vascular dimensions were related to an obstructive defect. VC, FEV and FEF$_{25-75}$, however, can be reduced in both obstruction and restriction and thus the correlations with markers of vascular dilation and reductions in VC, FEV$_1$ and FEF$_{25-75}$ may also indicate a relationship between vascular dilation and the development of restrictive lung function.
In this cohort, 23% of patients had evidence of a restrictive lung function defect and 17% had evidence of an obstructive defect. Those results are consistent with those of Santoli et al [133] but differ from those of Klings et al, [134] who found a much lower incidence of airflow obstruction. The differences may be due to a number of factors. This study used a recently reported ethnic-specific reference range for spirometry, [186] whereas Klings et al [134] used a Caucasian reference range with fixed correction factors to adjust for ethnicity. In this study abnormalities were classified based on the lower limit of normal based on percentiles recommended by the American and European Thoracic Societies, [136] whereas Klings et al used a fixed percentage predicted value to define the lower limit of normal for all lung function indices, which does not take into account that the limits of the normal range vary with age, sex and ethnicity [190]. Furthermore, the classification scheme in the Klings study specified that in order to be classified as obstructive or mixed, the DLCO had to be normal, which precluded the possibility that impaired gas transfer coexisted with airway abnormalities.

Restrictive lung function defects, that is, a reduced TLC and RV, were not associated with the extent of ground glass opacification or a reticular pattern and showed only a modest association with linear bands, suggesting interstitial fibrosis may not be the predominant mechanism for loss of lung volume in patients with SCD. Indeed, most CT markers of pulmonary fibrosis showed no association with reductions in VC, FEV₁, FEF₂₅/₇₅, RV:TLC SpO₂, Rs(0) or Rs(1). The extent of a reticular pattern was associated with a reduction in respiratory system resistance; it is reasonable to
speculate that this might be due to the tractional effects of areas of fibrosis on adjacent bronchi. Subpleural curvilinear bands were noted in 26% of patients; this is an unusual CT sign, formerly believed to be pathognomonic of early asbestosis [191]. Subpleural curvilinear bands have subsequently been described in association with micro-atelectasis in patients with atrial septal defect [192] or respiratory muscle weakness [193] and as reversible sign caused by interstitial oedema resulting from pulmonary congestion [194]. Observations in subjects without lung disease and undergoing lymphography have led to the suggestion that the sign may represent an engorged subpleural lymphatic network [195]. Given the presence of a high-output state in SCD, and the correlation of subpleural curvilinear lines with markers of small-vessel dilatation observed in this cohort, it is tempting to speculate that this pattern may be related to interstitial oedema and/or increased lymphatic drainage.

This study highlights a decline in lung function over a median of 6.6 years in adults with SCD. A significant decrease was observed in VC, FEV\textsubscript{1}, FEF\textsubscript{25-75}, FEV1:VC and KCOc with a significant increase in RV:TLC. There were no significant changes in the results of the CT assessments other than in vascular dimension assessments, where both the A/B ratio and CSA<5 mm% significantly increased. Those results indicate an association between changes in pulmonary vascular dimensions and the decline in lung function. Furthermore, a greater baseline CSA<5 mm% was predictive of a more rapid progression of both obstructive (increasing RV:TLC) and restrictive (decreasing TLC) lung disease and an increased likelihood of a deterioration in
parenchymal disease, as evidenced on HRCT examination, suggesting that the association observed between markers of pulmonary vascular dilatation may represent a causal relationship. Whilst TLC and RV did not decline significantly over the follow-up period for the group as a whole, these exploratory analyses suggest that there may be a degree of heterogeneity in the response to ageing and that a subgroup of patients do suffer a continued progression of restrictive disease. It may be of benefit, therefore, to test this hypothesis in a larger cohort. Our results emphasise the phenotypic heterogeneity of SCD lung disease. The changes in vascular morphology related to obstructive defects and to the likelihood of deteriorating interstitial lung disease, suggesting that there may be a shared mechanism involving small pulmonary vessels. Field et al [196] demonstrated that bone marrow-derived fibrocytes may be mobilised into the circulation and subsequently extravasate into the lungs of SCD mice. There they function as mesenchymal progenitor cells for the production of extracellular matrix and contribute to the development of fibrosis. Elevated levels of circulating fibrocytes have been observed in humans with SCD [196]. It is tempting, therefore, to speculate that pulmonary vascular engorgement and distension may potentiate extravasation of circulating fibrocytes.

This study has strengths, but some limitations too. A strength of this study was the use of two different quantitative methods for assessing pulmonary vascular morphology, the segmental A/B ratio and CSA<5 mm%, which yielded similar results. Ethnic-specific reference ranges were used for spirometric indices, but the
static lung volume results were related to reference ranges derived from Caucasian subjects with a fixed correction factor to account for ethnicity. All the study population, however, were African or Caribbean, thus correlations within the cohort and comparisons between results at baseline and follow-up were valid. A limitation is that subjects did not undergo right heart catheterisation to obtain pulmonary artery pressures and pulmonary vascular resistance, but all the patients underwent the same echocardiographic protocol. Studies have demonstrated a minority of patients with elevated TRV have elevated pulmonary artery pressure. In a recent study, only 8 of 26 patients with elevated TRV at echocardiography had elevated pulmonary artery pressure confirmed by right heart catheterisation [77] and similar findings were reported in a larger study with 10.4% of 243 patients with elevated TRV having pulmonary hypertension [197]. Different HRCT protocols were used in the earlier study [73], and in this study, however, care was taken to ensure that all the images used for comparisons were from anatomically comparable sections. In addition, scoring was undertaken by observers who were blinded to the results of lung function and echocardiography results. The consistent relationship between CSA<5 mm%, A/B ratio and lung function test results suggests the different protocols did not adversely influence the results. Furthermore, this study has shown that, if TLV\textsubscript{CT} is measured, volumetric HRCT scans are able to capture both restrictive and obstructive functional abnormalities, providing an alternative method to assess global pulmonary impairment in patients with SCD.
4.5 Conclusion

In conclusion, this study has demonstrated an association between small-vessel pulmonary vascular dimensions on HRCT reflecting pulmonary vascular volume, lung function abnormalities and echocardiographic estimates of CO and ventricular function in adults with SCD. These results suggest that abnormalities in pulmonary vascular volumes may explain some of the lung function abnormalities and the decline in lung function seen in adults with SCD.
5. AIRWAYS OBSTRUCTION AND PULMONARY CAPILLARY BLOOD VOLUME IN CHILDREN WITH SICKLE CELL DISEASE

Note: The twenty-five SCD patients in the bronchodilator subgroup were recruited by Dr Catherine Wedderburn (King’s College London), who assisted with testing. Lung function tests and statistical analyses were performed by the candidate.

5.1 Introduction

Obstructive lung function abnormalities appear to be common in children with SCD [139, 140, 145], but the aetiology is unclear. Dilation of the pulmonary vasculature resulting from the increased pulmonary capillary blood volume, which occurs in SCD patients as a result of chronic anaemia [123, 129, 130, 198], may increase peripheral airway obstruction. Inflation of the leg compartments of pneumatic trousers increases thoracic blood volume and increased respiratory resistance in healthy volunteers [124]. Acute intravascular volume expansion increased air flow obstruction in patients with left ventricular dysfunction [125]. In addition, in patients with impaired left ventricular function, pre-treatment with methoxamine a potent vasoconstrictor, fully prevented the methacholine induced decrease in forced expiratory volume in one second (FEV1) suggesting that the bronchoconstriction was mediated, at least in part, by vascular dilatation [128]. The aim of this study was to determine whether in children with SCD their increased pulmonary capillary blood volume was associated with airways obstruction. This
study, therefore, determined whether there were significant correlations between lung function tests and pulmonary capillary blood volume in children with SCD and age and ethnic matched controls. In a subset of subjects, measurements were repeated before after bronchodilator administration to assess whether any airflow obstruction observed was reversible, and whether any relationships between airflow obstruction and pulmonary capillary blood volumes were still present after bronchodilator.

5.2 Methods

Children with HbSS disease were recruited. Age and ethnic matched children without HbSS were recruited as controls; they were siblings of the SCD children or from local schools. Controls were recruited such that overall group characteristics were matched (Table 5.1) all control subjects were of a similar ethnic background to the children in the SCD group. Only children over seven years of age were recruited as they were likely to be able to complete the lung function tests. Respiratory system resistance, spirometry, body plethysmography and then pulmonary capillary blood volume were assessed. In a subset of SCD patients (n=25) and controls (n = 25), comprising the first twenty-five consecutive subjects recruited in each group, respiratory system resistance, spirometry and pulmonary capillary blood volume were measured before and 30 minutes after bronchodilator (ipratropium bromide) administration. An anticholinergic, ipratropium bromide,
was used as the bronchodilator as it has minimal cardiovascular side effects [199]. In the bronchodilator subgroup each subject was given eight puffs of ipratropium bromide via a metered dose inhaler and spacer device (20 μg per metered inhalation, i.e., total dose 160 μg) - the MDI was vigorously shaken between puffs and the delivery of each puff was followed by five normal breaths. Children in this subgroup were assessed pre and post-bronchodilator to determine whether any changes in response to the bronchodilator were greater in the SCD children and to evaluate whether any correlations observed between lung respiratory system resistance and pulmonary capillary blood volume remained after bronchodilation. For SCD children who underwent regular transfusions as part of their clinical care, lung function tests were performed immediately before their monthly transfusion to obviate any acute effects of blood transfusion on lung function. The study was approved by the King's College Hospital Research Ethics Committee and parents gave informed written consent for their child to take part.

5.2.1 Lung function assessments

FEV$_1$, vital capacity, forced expiratory flow between 25 and 75% of VC, total lung capacity, residual volume and respiratory system resistance were measured as previously described (Section 3). Since airways resistance can be altered by deep inspiration, $R_{rs}$ was measured before any of the other lung function tests. Furthermore, as the majority of the pulmonary blood volume is in the periphery of the lung [199], a resistance at 5 Hz was used ($R_{rs5}$), as low oscillation frequencies
have been shown to be transmitted more distally in the lung and therefore may reflect changes in peripheral airway calibre, [200] which are more likely to be influenced by an increased pulmonary capillary blood volume. The RrsS results were expressed as the percent predicted for height using the reference range of Nowoweijska et al [201]. The mean of two measurements within 5% of each other was reported.

Patients were diagnosed with an obstructive abnormality if their FEV1:FVC was less than the lower limit of normal (LLN), based on the ethnic specific reference range of Quanjer et al [186]. A restrictive abnormality was diagnosed if the TLC was below the LLN based on the reference range of Rosenthal et al [202], with a –12% correction factor applied [136]. A mixed abnormality was diagnosed if the TLC and FEV1:VC were less than the LLNs.

5.2.2 Pulmonary capillary blood volume

Pulmonary capillary blood volume was measured using the single breath-hold method for gas transfer for carbon monoxide (DLCO) and nitric oxide (DLNO) as previously described. Pulmonary capillary blood volume was expressed as $V_c$ per unit alveolar volume ($V_c/VA$). The mean of two measurements within 5% was reported. Results were corrected for haemoglobin concentration [Hb].

5.2.3 Haematological data
Haemoglobin, lactate dehydrogenase (LDH), bilirubin levels and reticulocyte counts were obtained from routine blood tests taken within one month of testing when patients were clinically stable. For the control subjects, Hb was measured non-invasively using Hb-pulse oximetry with a paediatric probe (Masimo Radical 7 + rainbow probe, Masimo, CA).

5.2.4 Sample size

For the bronchodilator sub-analysis, comparison of 25 children in each group allowed detection of a change in respiratory system resistance at 5Hz of 0.31kPa/l/sec (one standard deviation of the normative data of Tomalak et al. [203]) between the groups and before and after bronchodilator administration with 90% power at the 5% level.

5.2.5 Statistical analysis

Data were tested for normality using the D'Agostino and Pearson omnibus normality test. Since data were not normally-distributed, comparisons were made with Mann-Whitney U tests.
5.3 Results

5.3.1 Subjects

Sixty-seven children with SCD and thirty-six controls were tested. Testing this number of patients allowed the relationships between spirometric measures of airflow obstruction to additionally be assessed reliably as these measurements are less sensitive than respiratory system resistance. The SCD children and the controls were of similar age, but the median haemoglobin levels of the SCD children were significantly lower than that of the controls (P < 0.0001; Table 5.1). There was a trend towards a greater proportion of girls in the control compared to the SCD group, although this did not reach statistical significance.

<table>
<thead>
<tr>
<th></th>
<th>SCD children</th>
<th>Controls</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>67</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>13.4 (7.4–18.2)</td>
<td>14.4 (7.4–18.0)</td>
<td>0.2247†</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>36 (54)</td>
<td>12 (33)</td>
<td>0.0627‡</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>8.2 (6.1–10.9)</td>
<td>12.5 (10.1–15.0)</td>
<td>&lt;0.0001†</td>
</tr>
</tbody>
</table>

Table 5-1 Demographics by SCD status.
The results are expressed as n (%) or median (range). †Wilcoxon signed-rank test. ‡Fisher’s exact test.
Sixteen SCD children (24%) had a physician diagnosis of asthma (clinician documented asthma in the medical records) and twenty-two (33%) had had at least one acute chest syndrome (ACS) episode. Ten SCD children (15%) were receiving hydroxyurea treatment and sixteen (24%) were undergoing regular transfusions. Four of the controls had a physician’s diagnosis of asthma (11%), this did not differ significantly to the proportion of SCD children with a physician’s diagnosis of asthma (p=0.1904).

In the SCD group, 11 children (16%) had an obstructive, 10 (15%) a restrictive and three (4%) a mixed abnormality. Fourteen SCD children (21%) had evidence of airflow obstruction; i.e., had an obstructive or mixed obstructive/restrictive abnormality. In the control group three children (8%) had an obstructive and none a mixed restrictive pattern. Both obstructive and restrictive abnormalities were significantly more common in the SCD group compared to the controls (p = 0.0479, p = 0.0035 respectively).

5.3.2 Lung function and pulmonary capillary blood volume

The SCD children compared to the controls had significantly higher median RrsS (p < 0.0001), RV:TLC (p=0.022) and KCOc (p = 0.0082) and lower FEV₁ (p < 0.0001), VC (p < 0.0001), FEV₁:VC (p = 0.0116), FEF²⁵⁻⁷⁵ (p < 0.0001), TLC (p < 0.0001) (Table 5.2). Pulmonary capillary blood volume was significantly higher in the SCD children (p < 0.0001) (Table 5.2, Figures 5.2 – 5.4).
<table>
<thead>
<tr>
<th>LFT</th>
<th>SCD children (n=67)</th>
<th>Controls (n=36)</th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td>(%predicted)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rrs5</td>
<td>123.1 (77.1-204.5)</td>
<td>98.8 (64.3-155.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FEV₁</td>
<td>82.0 (54.8-139.6)</td>
<td>104.4 (73.4-131.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>VC</td>
<td>89.5 (58.2-142.1)</td>
<td>106.0 (80.2-140.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FEV₁:VC</td>
<td>93.9 (70.0-108.8)</td>
<td>99.4 (74.5-112.8)</td>
<td>0.0116</td>
</tr>
<tr>
<td>FEF₂₅-₇₅</td>
<td>63.1 (22.5-127.0)</td>
<td>102.3 (37.5-160.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TLC</td>
<td>90.3 (63.1-135.2)</td>
<td>101.0 (84.5-125.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RV</td>
<td>86.0 (31.8-131.2)</td>
<td>89.8 (55.1-155.3)</td>
<td>0.3667</td>
</tr>
<tr>
<td>RV:TLC</td>
<td>114.8 (49.9-179.2)</td>
<td>108.3 (69.1-157.5)</td>
<td>0.2275</td>
</tr>
<tr>
<td>DLCOc</td>
<td>90.3 (64.9-138.3)</td>
<td>91.0 (66.1-127.7)</td>
<td>0.6756</td>
</tr>
<tr>
<td>KCOc</td>
<td>94.8 (75.9-140.2)</td>
<td>87.6 (77.8-115.1)</td>
<td>0.0082</td>
</tr>
<tr>
<td>Vc/Vₐ* (ml/L)</td>
<td>24.9 (14.0 - 50.5)</td>
<td>18.2 (12.2 - 28.2)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 5-2 Lung function and pulmonary capillary blood volume data by SCD status.

The results are expressed as median (range) and percent predicted for height except where indicated *.

†Mann-Whitney U test.
Figure 5-1 Impulse oscillometry and spirometry in SCD and control children.

Data are presented as percentage of predicted for height and sex. The red point indicates the median value.
Figure 5-2 Lung volumes in SCD and control children.

Data are presented as percentage of predicted for height and sex. The red point indicates the median value.
Figure 5-3 Gas transfer and pulmonary capillary blood volume in SCD and control children.

Data are presented as percentage of predicted for height and sex except where indicated. The red point indicates the median value.
5.3.3 Relationships between pulmonary function and pulmonary capillary blood volume

There was a significant positive correlation in the SCD children between pulmonary capillary blood volume and Rrs5 (p < 0.0001) and RV:TLC (p = 0.0009) and a significant negative correlation with FEV₁ (p = 0.0015), VC (p =0.0169) and FEF₂₅-₇₅ (p = 0.0062) (Table 5.3). In the controls, there were no significant correlations between pulmonary capillary blood volume and any of the lung function tests (Table 5.4). Figure 5.1 shows the relationship between Rrs5 and pulmonary capillary blood volume in the SCD and control children.

In the SCD children the pulmonary capillary blood volume was significantly correlated with haemoglobin concentration (r = - 0.459, p < 0.0001), but not with LDH (r = 0.05, p = 0.7025) or reticulocyte count (r = 0.760, p = 0.0939).
<table>
<thead>
<tr>
<th></th>
<th>r†</th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rrs5</td>
<td>0.500</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FEV₁</td>
<td>-0.390</td>
<td>0.0029</td>
</tr>
<tr>
<td>VC</td>
<td>-0.291</td>
<td>0.0010</td>
</tr>
<tr>
<td>FEV₁:VC</td>
<td>-0.201</td>
<td>0.1011</td>
</tr>
<tr>
<td>FEF₂₅₋₇₅</td>
<td>-0.433</td>
<td>0.0062</td>
</tr>
<tr>
<td>TLC</td>
<td>-0.160</td>
<td>0.1187</td>
</tr>
<tr>
<td>RV:TL  C</td>
<td>0.470</td>
<td>0.0009</td>
</tr>
<tr>
<td>RV</td>
<td>0.220</td>
<td>0.0739</td>
</tr>
</tbody>
</table>

Table 5-3 Correlations between pulmonary capillary blood volume and lung function results in the SCD children.

†Spearman’s rank correlation.
<table>
<thead>
<tr>
<th>LFT</th>
<th>r†</th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td>( R_{v5}S )</td>
<td>-0.120</td>
<td>0.4920</td>
</tr>
<tr>
<td>FEV(_1)</td>
<td>-0.231</td>
<td>0.1735</td>
</tr>
<tr>
<td>VC</td>
<td>-0.230</td>
<td>0.1845</td>
</tr>
<tr>
<td>FEV(_1):VC</td>
<td>-0.081</td>
<td>0.6566</td>
</tr>
<tr>
<td>FEF(_{25/75})</td>
<td>-0.193</td>
<td>0.2718</td>
</tr>
<tr>
<td>TLC</td>
<td>-0.02</td>
<td>0.8874</td>
</tr>
<tr>
<td>RV:TLC</td>
<td>0.343</td>
<td>0.4360</td>
</tr>
<tr>
<td>RV</td>
<td>0.333</td>
<td>0.0501</td>
</tr>
</tbody>
</table>

Table 5-4 Correlations between pulmonary capillary blood volume and lung function results in the control children.

†Spearman’s rank correlation.
Figure 5-4  Rs5 and pulmonary capillary blood volume (Vc/VA) in the SCD (red points) and control subjects (blue points).

Lines represent the least-squares linear regression best fit in each group; the shaded areas are the 95% confidence intervals for the fit.
### 5.3.4 Bronchodilator response

The SCD children in the subgroup who had post-bronchodilator measurements of respiratory system resistance and pulmonary capillary blood volume, compared to the controls, before and after bronchodilator administration, had a higher median $R_{rs5}$ ($p=0.0046$, $p=0.0419$ respectively) and a higher median $Vc/VA$ ($p<0.0001$, $p<0.0001$ respectively) (Table 5.5). Both groups had a significant decrease in $R_{rs5}$ post bronchodilator ($p<0.0001$ SCD children, $p<0.0001$ controls), but there was no significant difference in the magnitude of the reduction in $R_{rs5}$ post-bronchodilator between the SCD children and the controls ($p=0.090$) (Table 5.5). There were no significant changes in $Vc/VA$ post-bronchodilator in either group ($p=0.484$ SCD children; $p=0.514$ controls) and no significant difference in the magnitude of change between the two groups ($p=0.090$) (Table 5.5). Four of the SCD children and two of the controls had a significant response to bronchodilator based on FEV$_1$.

In the SCD children, there were significant correlations between $R_{rs5}$ and $Vc/VA$ both pre-bronchodilator ($r=0.540$, $p=0.005$) and post-bronchodilator ($r=0.482$, $p=0.015$). In the controls, there were no significant correlations between $R_{rs5}$ and $Vc/VA$ either pre-bronchodilator ($p=0.319$) or post bronchodilator ($p=0.345$).
<table>
<thead>
<tr>
<th></th>
<th>SCD children</th>
<th>Controls</th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>25</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td><strong>Pre bronchodilator:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rs5</td>
<td>132.7 (87.7 – 181.1)</td>
<td>102.4 (83.3 – 184.2)</td>
<td>0.0046</td>
</tr>
<tr>
<td>Vc/VA (ml/l)</td>
<td>25.7 (18.2 – 38.4)</td>
<td>18.4 (13.9 – 25.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Post bronchodilator:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rs5</td>
<td>105.0 (78.7 – 150.0)</td>
<td>91.3 (64.3 – 147.4)</td>
<td>0.049</td>
</tr>
<tr>
<td>Vc/VA (ml/l)</td>
<td>25.8 (18.5 – 41.9)</td>
<td>17.7 (13.1 – 27.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Change in Rs5 post-bronchodilator (%)</strong></td>
<td>-18.5 (-36.0 to -4.6)</td>
<td>-12.5 (-28.9 to 5.6)</td>
<td>0.090</td>
</tr>
<tr>
<td><strong>Change in Vc/VA post-bronchodilator (%)</strong></td>
<td>-1.6 (-8.4 to 6.1)</td>
<td>-2.4 (-6.3 to 2.0)</td>
<td>0.587</td>
</tr>
</tbody>
</table>

Table 5-5 Peripheral airways resistance and pulmonary capillary blood volume pre- and post-bronchodilator according to SCD status.

The results are expressed as median (range) and percent predicted for height. †Mann-Whitney U test.
5.4 Discussion

This study has demonstrated that SCD children compared to ethnic origin matched controls had higher respiratory system resistance and pulmonary capillary blood volume both before and after bronchodilator. Respiratory system resistance and pulmonary capillary blood volume results were significantly correlated in the SCD children, but not in the controls, suggesting the raised respiratory system resistance in the SCD children was, at least partly, explained by their increased pulmonary capillary blood volume. Furthermore, pulmonary capillary blood volume correlated with the other measures of airway obstruction. In addition, a significant negative correlation was observed correlation between the pulmonary capillary blood volume and vital capacity. The raised pulmonary capillary blood volume seen in the SCD children is a response to their chronic anemia resulting in a raised cardiac output and increased pulmonary blood volume and vascular recruitment [123, 198]. The increase in pulmonary blood volume resulting from the increase in cardiac output due to their chronic anemia may explain the relatively normal DLCO results we report despite lower lung volumes. These results are consistent with raised pulmonary capillary blood volume reported in adults with SCD [130]. The significant correlation with between pulmonary capillary blood volume and the hemoglobin levels emphasizes the relationship between the levels of anaemia, pulmonary capillary blood volume and peripheral airway obstruction.

The SCD children had a significantly higher mean Rrs5 than the controls indicating they had elevated peripheral airways resistance and a greater prevalence of
obstructive abnormalities, findings consistent with other studies showing obstructive abnormalities in SCD children [139, 140, 145, 204]. Spirometry and lung volume measurements demonstrated that the SCD children compared to the controls had significantly greater obstructive and restrictive abnormalities.

In the bronchodilator subgroup, whilst we observed a statistically significant change in lung function following bronchodilator administration, magnitude of change in respiratory system resistance in the SCD group was similar to that seen in the control children. Given that a degree of bronchial tone occurs even in healthy individuals, it is not surprising that a decrease in Rrs5 was seen in both SCD and controls after bronchodilator administration. These data indicate that the obstructive abnormalities observed in children with SCD are associated with their increased pulmonary capillary blood volume, rather than solely due to reactive airways disease.

Key strengths of this study are that comparison was made of SCD children to ethnic matched controls that were of similar age, and that an anticholinergic bronchodilator, ipratropium bromide, was used to assess bronchodilator response. Ipratropium bromide has small and clinically unimportant hemodynamic effects [199], and would, therefore, avoid the potential effects on cardiac output of β2-agonists which might impact pulmonary capillary blood volume. Indeed, no change in pulmonary capillary blood volume was seen after bronchodilator administration. Impulse oscillometry was used to assess respiratory system resistance, as this technique has been found to be more sensitive in assessing peripheral airway
function than spirometry [200]. The respiratory system resistance results were related to reference ranges derived from Caucasian subjects with [201], but the two groups were matched for ethnic origin and the same reference ranges were used in both groups, thus comparisons between them were valid. The correlations between impulse oscillometry results and pulmonary capillary blood volume were supported by spirometric measures of airway obstruction also correlating with pulmonary capillary blood volume. Blood samples were not taken to assess hemoglobin levels in the controls, but the levels determined using the Hb pulse oximetry were within the normal range, as would be expected in healthy controls. In addition, non-invasive measurement of hemoglobin concentration has been validated in healthy adult volunteers undergoing haemodilution [205] and in adult surgical and critical care patients [206, 207].

5.5 Conclusion

In conclusion, these results suggest that the raised pulmonary capillary blood volume seen in SCD children may, at least partially, explain their increased airways resistance. Strategies to reduce anaemia and increased pulmonary capillary blood volume, such as hydroxyurea or blood transfusion, may be beneficial in those who remain symptomatic despite optimization of bronchodilator therapy and merits testing.
6. THE ACUTE EFFECT OF BLOOD TRANSFUSION ON AIRWAYS OBSTRUCTION AND PULMONARY CAPILLARY BLOOD VOLUME IN CHILDREN WITH SICKLE CELL DISEASE

Note: All lung function tests and statistical analyses were performed by the candidate.

6.1 Introduction

Children and adults with SCD have an increased pulmonary capillary blood volume which is a response to their chronic anemia resulting in a raised cardiac output and increased vascular recruitment and distension [123, 129, 130, 198, 208, 209].

Experimental increases in thoracic blood volume have been shown to increase airways resistance. Rapid saline infusion has been shown to produce a significant decrease in $\text{FEV}_1$ in healthy adults [126, 127] as well as in patients with left ventricular failure [125]. Lorino et al found that the inflation of pneumatic trousers in healthy subjects caused an increase in oscillometric resistance [124]. Additionally, acute increases in thoracic blood volume have been shown to increase sensitivity to methacholine [210] in healthy adults suggesting a role for vascular expansion in reactive airways disease. Such studies have not, however, been performed for obvious reasons in SCD children.
Some SCD children receive transfusion as part of routine care [211], hence this treatment would allow a natural experiment to investigate whether fluid loading with consequent transient increase in cardiac output [127] worsened airflow obstruction in SCD. Demonstration of such an effect would support the hypothesis that anaemia and increased pulmonary capillary blood volume might be responsible for the increased airways obstruction and wheezing seen in SCD populations. The aim of this study therefore was to test the hypothesis that transfusion would result in an acute increase in pulmonary capillary blood volume and a worsening of airflow obstruction in children with SCD.

6.2 Methods

Children with HbSS disease undergoing regular blood transfusion at King’s College Hospital, London were recruited. Only children six years of age or greater were recruited as they were likely to be able to complete the lung function tests. Impulse oscillometry, spirometry, and pulmonary capillary blood volume were measured before and immediately after transfusion. In order to further characterise the patients, static lung volumes were measured before transfusion. The volume of packed red cells (PRCV) administered to each patient was determined using the following formula: 

$$\text{PRCV (mls/kg)} = \text{patient weight (kg)} \times \text{change in haemoglobin to be achieved (g/dl)} \times \text{transfusion factor (4)}$$

in order to achieve a target haemoglobin concentration of 13.5g/dl. All transfusions were administered over an interval of approximately four hours. The study was approved
by the King’s College Hospital Research Ethics Committee and parents gave informed written consent for their child to take part.

6.2.1 Lung function assessments

FEV$_1$, VC, forced expiratory flow between 25 and 75% of VC, total lung capacity, residual volume and respiratory system resistance were measured as previously described (Section 3). As before, R$_{rs}$ was measured before any of the other lung function tests, and a resistance at 5 Hz was used (Rrs5) to capture changes in small airway function. The Rrs5 results were expressed as the percent predicted for height using the reference range of Nowowiejska et al [201]. The mean of two measurements within 5% of each other was reported.

Patients were diagnosed with an obstructive abnormality if their FEV$_1$:FVC was less than the lower limit of normal (LLN), based on the ethnic specific reference range of Quanjer et al [186]. A restrictive abnormality was diagnosed if the TLC was below the LLN based on the reference range of Rosenthal et al [202], with a −12% correction factor applied [136]. A mixed abnormality was diagnosed if the TLC and FEV$_1$:VC were less than the LLNs.
6.2.2 Pulmonary capillary blood volume

Pulmonary capillary blood volume was measured using the single breath-hold method for gas transfer for carbon monoxide (DLCO) and nitric oxide (DLNO) as previously described. Pulmonary capillary blood volume was expressed as \( V_c \) per unit alveolar volume (\( V_c/VA \)). The mean of two measurements within 5% was reported. Results were corrected for haemoglobin concentration [Hb] from a blood test taken immediately before transfusion.

6.2.3 Sample size

18 children studied pre- and post-transfusion allowed detection of a change in respiratory system resistance at 5Hz of 0.31kPa/l/sec (one standard deviation of the normative data of Tomalak et al. [203]) before and after transfusion administration with 80% power at the 5% level.

6.2.4 Statistical analysis

Data were tested for normality using the D’Agostino and Pearson omnibus normality test. Since data were not normally-distributed, paired analyses were performed using paired-sample Wilcoxon matched-pairs signed rank tests for pre- and post- transfusion comparisons.
6.3 Results

6.3.1 Subjects

Eighteen subjects with a median age of 14.2 (range 6.6-18.5) years were assessed, seven of whom (39%) were female. Their median haemoglobin was 10.1 (range 8.2 – 11.6) g/dl, and PRVC 10.8 (5.9 – 16.4) ml/kg. Eight children (44%) had had at least one episode of ACS and four (22%) had a physician diagnosis of asthma.

6.3.2 Lung function and pulmonary capillary blood volume

Prior to transfusion, six children had an obstructive (33%) and two (11%) a restrictive abnormality. Pulmonary capillary blood volume, Rrs(5), D\textsubscript{L}COc and KCOc all increased significantly after transfusion (p <0.0001, p <0.0001, p =0.0001 respectively), whereas FEV\textsubscript{1}, VC, and FEF\textsubscript{25-75} all declined significantly (p = 0.0056, p = 0.0008, p = 0.0483). No significant change was seen in FEV\textsubscript{1}:VC (p = 0.2462) (Table 6.1) (Figure 6.1 and 6.2). The percentage change from baseline in pulmonary capillary blood volume was significantly correlated with that in Rrs5 (r = 0.61, p = 0.0129) (Figure 6.3), VC (r = -0.53, p = 0.0362) (Figure 6.3) and KCO (r = 0.59, p = 0.0216) (Figure 6.3) but not with FEV\textsubscript{1} (r = -0.14, p = 0.6174 ), FEF\textsubscript{25-75} (r = -0.31, p =0.0834), FEV\textsubscript{1}:VC (r = 0.07, p = 0.7950) or D\textsubscript{L}CO (r = 0.21, p = 0.4331).
<table>
<thead>
<tr>
<th></th>
<th>Pre-transfusion (n=18)</th>
<th>Post-transfusion (n=18)</th>
<th>%change from baseline</th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{c}/V_{A}*$ (ml/L)</td>
<td>21.6 (13.6 – 47.3)</td>
<td>28.2 (14.9 – 57.1)</td>
<td>25.2 (5.4-106.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rrs5</td>
<td>127.4 (88.9-207.8)</td>
<td>141.3 (96.1-234.1)</td>
<td>10.7 (-3.6 - 22.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FEV$_1$</td>
<td>84.4 (66.2-139.6)</td>
<td>79.4 (63.5-132.9)</td>
<td>-4.9 (-20.5 - 8.6)</td>
<td>0.0056</td>
</tr>
<tr>
<td>VC</td>
<td>94.2 (73.7-142.1)</td>
<td>90.72 (60.5-144.0)</td>
<td>-5.5 (-9.8 - 6.1)</td>
<td>0.0008</td>
</tr>
<tr>
<td>FEV$_1$:VC</td>
<td>91.2 (73.0-105.3)</td>
<td>92.7 (74.5-112.8)</td>
<td>-2.6 (-12.0 –17.5)</td>
<td>0.2462</td>
</tr>
<tr>
<td>FEF$_{25-75}$</td>
<td>59.9 (34.4-122.5)</td>
<td>62.5 (30.3-111.5)</td>
<td>-8.9 (-55.8 - 45.6)</td>
<td>0.0483</td>
</tr>
<tr>
<td>DLCOc</td>
<td>88.3 (76.0-122.6)</td>
<td>108.9 (81.2-145.5)</td>
<td>10.7 (1.0 - 27.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>KCOc</td>
<td>86.8 (75.1-109.3)</td>
<td>105.9 (80.5-123.8)</td>
<td>17.0 (-3.8 -3 5.7)</td>
<td>0.0001</td>
</tr>
<tr>
<td>TLC</td>
<td>92.2 (71.6-135.1)</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV</td>
<td>90.1 (61.7-104.3)</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV:TLC</td>
<td>105.1 (87.4-134.8)</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6-1  Lung function and pulmonary capillary blood volume before and after transfusion.

The results are expressed as median (range) and percent predicted for height except where indicated *.

†Wilcoxon signed-rank test.
Figure 6-1  Rs5, FEV₁, VC and FEV₁:VC before and after transfusion. Individual data are shown by linked data points.
Figure 6-2 FEF_{25-75}, D_{L}CO and V_{L}/VA before and after transfusion. Individual data are demonstrated by linked data points.
Figure 6-3 Change in $V_{l}/VA$ and lung function after transfusion.

Lines represent the least-square linear regression best fit in each group, the shaded areas are the 95% confidence interval for the fit.
6.4 Discussion

This study has demonstrated significant changes in lung function immediately following blood transfusion in children with SCD. There was an increase in respiratory system resistance and gas transfer with corresponding decreases in FEV\(_1\) and VC. Pulmonary capillary blood volume also increased significantly, and this change was correlated with the change in Rrs5, VC and KCO.

The increase in Rrs5 and decrease in FEV\(_1\) are consistent with worsening airflow obstruction following fluid loading by transfusion. The VC also decreased, resulting in no significant change in FEV\(_1\):VC. Whilst this might suggest a role for increased pulmonary capillary blood volume in the aetiology of restrictive abnormalities in SCD, given that no significant correlation was found between pulmonary vascular volumes and TLC in both adults and children with SCD in previous studies (see Chapters 4 and 5 of this thesis) it seems more likely that this represents loss of VC due to hyperinflation as a consequence of increasing small airways obstruction [208, 209]. Those results reinforce the association between elevated pulmonary capillary blood volume and obstructive abnormalities in children with SCD.

Compared to healthy controls, children with SCD have an elevated cardiac output and pulmonary blood flow at rest, as well as raised pulmonary capillary blood volume as a result of chronic anaemia [198, 212]. On exercise, both pulmonary blood flow and pulmonary capillary blood volume increase with workload in SCD.
children and controls [212]. In children with SCD, however, the increase in pulmonary blood flow relative to pulmonary capillary blood volume is disproportionately high compared to control subjects, indicating a lack of pulmonary vascular reserve [212] due to the fact that the pulmonary capillary vessels are already over-distended at rest [198]. This baseline distension may make children with SCD particularly sensitive to any further volume load, resulting perhaps in mechanical compression of the peripheral airways. This may explain that, although the rate of infusion in this study was much slower than that in the study of Puri et al in patients with left heart failure (four hours versus thirty minutes) for a similar total fluid volume in ml/kg, the change in FEV$_1$ was comparable [125]. An increase in respiratory system resistance in healthy subjects in response to intravenous infusion of normal saline has also been demonstrated [126, 127]. These results suggest a causal link between increased pulmonary vascular volume and airflow obstruction in SCD, and that it is possible wheezing reported in some children with SCD may have been incorrectly attributed to asthma.

This study has some strengths and some limitations. The children who participated in the study all undergo regular transfusions as part of their clinical management, this means that they are, in general, likely to suffer from more severe disease and may, therefore, be at greater risk of SCD-related vasculopathy [48, 52, 53]. Such children may have early pulmonary vasculopathy and this may have modulated their response to fluid loading. It would, however, be inappropriate to administer a
fluid load to an unselected cohort of SCD children. Hence, investigating SCD children following a transfusion represents an appropriate methodology to study the effects of this potential disease mechanism. Haemoglobin levels take approximately 24 hours to stabilise after transfusion, hence the pre-transfusion Hb level was used to correct the DlCO measurement from which pulmonary capillary blood volume was derived, which may have resulted in an overestimation of the change in pulmonary capillary blood volume. The median haemoglobin level in this cohort, however, was relatively high (10.1 g/dl) and therefore the error introduced by correcting for haemoglobin concentration was likely to have been small [131]. Indeed the change in pulmonary capillary blood volume correlated with both the change in Rrs5 and VC neither of which are influenced by the haemoglobin correction. A difficulty in the interpretation of these data is the lack of a control group. It would, however, not be ethically acceptable to administer blood transfusions or intravenous fluids to children in whom there was no medical requirement to do so. The respiratory system resistance results were related to reference ranges derived from Caucasian subjects [201], but since the same reference range was used for both pre- and post-transfusion measurements, paired comparisons between them were valid. Static lung volumes were not measured post-transfusion in order to minimize the time burden on the participants, and therefore, it is not possible to rule out an effect of blood transfusion on pulmonary restriction. No association between TLC and measures of pulmonary vascular volume were, however, seen in two previously described studies (chapter four and five).
6.5 Conclusion

This study has demonstrated that significant increases in pulmonary capillary blood volume and airways resistance occur immediately following blood transfusion in children with SCD. These changes are accompanied by significant reductions in FEV$_1$, VC and FEF$_{25-75}$. Furthermore, the change in lung function correlated with the increase in pulmonary capillary blood volume.
7. EXHALED NITRIC OXIDE AND PULMONARY BLOOD FLOW IN CHILDREN WITH SICKLE CELL DISEASE

Note: All lung function tests and statistical analyses were performed by the candidate.

7.1 Introduction

Measurement of exhaled nitric oxide ($f_{eNO}$), which reflects airway NO production and is a marker for eosinophilic airways inflammation, might help to further elucidate whether asthma is increased in SCD children as $f_{eNO}$ is often elevated in patients with asthma [120]. Early studies suggested that $f_{eNO}$ was reduced in SCD patients [121], which might reflect the reduced nitric oxide (NO) bioavailability due to scavenging by cell free haemoglobin resulting from chronic haemolysis [213]. A reduced $f_{eNO}$, however, has not been found in other cohorts assessed more recently [204, 214]. $f_{eNO}$ has been previously measured at a single fixed exhalation flow of 50ml/s, but if $f_{eNO}$ is measured at multiple exhalation flows, it can be partitioned into flow-independent airway and alveolar components [171, 172]. Using such a technique, children with SCD 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 [214]. The authors, therefore, suggested that
subclinical airway inflammation might occur in SCD patients [214]. A limitation of that study, however, was that the children in the control group were not matched for race and ethnic differences have been reported for \( f_{eNO} \) indices [215-217]. Furthermore, as the transfer factor for NO (D\(_L\)NO) was not measured [13], it was not possible from that study to determine the relative importance of NO production (as opposed to concentration, which may change as a results of changes in endogenous production or by accumulation due to impaired transfer of NO). Thus, it is important that flow independent \( F_{eNO} \) in SCD patients is compared with appropriately matched controls to reliably determine whether or not \( F_{eNO} \) is elevated in SCD children. Exhaled NO has been shown to be elevated in patients with atrial septal defects or liver cirrhosis; both conditions are associated with a hyper-dynamic pulmonary circulation and \( f_{eNO} \) correlated with increased pulmonary blood flow [122, 218]. Children with SCD often have a hyper-dynamic circulation due to the raised cardiac output resulting from chronic anaemia [123, 198], but whether that influences exhaled NO in SCD patients has not been investigated. The aim of this study, therefore, was to test the hypotheses that airway and alveolar nitric oxide production would differ between children with SCD and ethnic-matched controls and that alveolar NO production was related to a hyperdynamic pulmonary circulation and/or airways obstruction as assessed by respiratory system resistance and FEV\(_1\):VC.
7.2 Methods

Afro-Caribbean children with HbSS disease were recruited. Age and ethnic matched children, who were siblings of the SCD children or from local schools, were recruited as controls. Controls were recruited such that overall group characteristics were matched (Table 7.1) all control subjects were of a similar ethnic background to the children in the SCD group. Only children over seven years of age were recruited as they were likely to be able to complete all the lung function tests. No child underwent testing within two weeks of an upper respiratory tract infection or an SCD child within a month of suffering a vaso-occlusive crisis. The study was approved by the King's College Hospital Research Ethics Committee and parents gave informed written consent for their child to take part.

7.2.1 Lung function assessments

FEV₁, VC, FEF₂₅₋₇₅, TLC, RV, DLCO and respiratory system resistance were measured as previously described (Section 3). Rrs was measured before any of the other lung function tests and a resistance at 5 Hz was used (Rrs₅) to capture changes in small airway function. The Rrs₅ results were expressed as the percent predicted for height using the reference range of Nowowiejska et al [201]. Exhaled NO was measured before the remaining lung function tests were performed as recent forced expiration can influence FeNO. Spirometry, static lung volumes and gas
transfer measurements were expressed as the percent predicted for height [186, 202]. Ethnic-specific reference equations were not available for static lung volumes or gas transfer, therefore the predicted values were adjusted using appropriate correction factors [219]. The transfer factor for nitric oxide ($D_L$NO) was obtained by the addition of 40ppm NO to the inspired gas mixture prior to commencing the single-breath DLCO measurement [167]. Patients were diagnosed with a restrictive abnormality if their TLC was less than the lower limit of normal (LLN) with a normal FEV$_1$;VC, an obstructive abnormality if their FEV$_1$;VC was less than LLN and a mixed pattern if both TLC and FEV$_1$;VC were less than the LLN [136].

7.2.2 Exhaled nitric oxide measurements

Exhaled nitric oxide was measured at multiple flow rates 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 [171] as described in chapter three. The equation of Perillo et al [220] was used to derive the rate of alveolar NO production ($V'_{A,NO}$) (nl/min) as follows:

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

Where $P_{H2O}$ is water vapour partial pressure (assumed to be 47mmHg) and $P_{atm}$ is the atmospheric pressure and $D_L$NO is expressed in ml/min/mmHg. Exhaled NO measurements were performed before the $D_L$CO-$D_L$NO manoeuvre to avoid contamination by inspired NO in the test gas mix.
7.2.3 **Pulmonary blood flow measurements**

Effective pulmonary blood flow ($Q_{\text{pulm}}$) was measured noninvasively by the inert-gas rebreathing method as previously described. The inspired gas mixture contained 0.5% nitrous oxide ($N_2O$) and 0.1% sulphur hexafluoride ($SF_6$) as the soluble and insoluble components respectively. To adjust for body size, the pulmonary blood flow index ($Q_{I_{\text{pulm}}}$) was derived by dividing $Q_{\text{pulm}}$ by the predicted body surface area using the formula of Mosteller [175].

7.2.4 **Statistical analysis**

Data were tested for normality using the D'Agostino and Pearson omnibus normality test. Since data were not normally-distributed, comparisons were made with Mann-Whitney U tests.

7.2.5 **Sample size**

Comparison of 18 children in each group allowed detection of a difference in the alveolar concentration of nitric oxide of one ppb (one standard deviation of the data of Linn *et al.* [217] between the groups with 90% power at the 5% level.
7.3 Results

7.3.1 Subjects

Eighteen children with SCD and eighteen healthy controls were assessed. The SCD children and the controls were of similar age and sex (Table 7.1). Two patients with SCD and one control had a physician diagnosis of asthma. One of the two children with SCD who had asthma had an elevated Fe\textsubscript{NO,50/4} (>35ppb) [221].

<table>
<thead>
<tr>
<th></th>
<th>SCD children</th>
<th>Controls</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>18</td>
<td>n=18</td>
<td></td>
</tr>
<tr>
<td>Sex (Female n (%))</td>
<td>9(50%)</td>
<td>9(50%)</td>
<td>1.0000‡</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>16.0 (9 – 18)</td>
<td>17.0 (11 – 18)</td>
<td>0.2310‡</td>
</tr>
<tr>
<td>[Hb] (g/dl)</td>
<td>8.3 (6.0 – 10.3)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Hydroxyurea n (%)</td>
<td>5 (27%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Transfusion n (%)</td>
<td>0.05%</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Table 7-1 Patient demographics by SCD status. †Fisher’s exact test. ‡Mann-Whitney U test.
7.3.2 Lung function, exhaled nitric oxide and pulmonary blood flow

Compared to the controls, the SCD children had a significantly lower FEV1 (p<0.0001), VC (p<0.0001), FEF_{25-75} (p=0.0071), FEV1:VC (p=0.0315), TLC (p=0.0024), and SpO2 (p=0.0023) and a significantly higher Rrs5 (p=0.0008) and KCO (p =0.0114) (Table 7.2). 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 control subjects had a restrictive defect and one an obstructive pattern. One control subject had an isolated reduction in DLCO.

There were no significant differences between the groups in F_{eNO, 50ml/s} or total maximal airway NO flux (J’_{aw,NO}) (Table 7.3). 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 (Q_{I pulm}) (p<0.0001).

In the SCD group, there was no significant difference in F_{eNO,50ml/s}, J’_{aw,NO}, or C_{A,NO} (p=0.5663, p=0.1433 and p=0.3877, respectively) in the patients on hydroxyurea treatment, although caution must be exercised when interpreting these results due to the small size of the hydroxyurea group.
<table>
<thead>
<tr>
<th></th>
<th>SCD children</th>
<th>Controls</th>
<th>p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=18</td>
<td>n=18</td>
<td></td>
</tr>
<tr>
<td>Rrs5</td>
<td>145.6(107.8-234.1)</td>
<td>111.7(67.2-168.6)</td>
<td>0.0008</td>
</tr>
<tr>
<td>FEV₁</td>
<td>79.1(47.5-105.8)</td>
<td>104.5(78.6-124.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>VC</td>
<td>85.1(48.6-107.8)</td>
<td>107.5(80.2-125.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FEF₂₅₋₇₅</td>
<td>58.5(29.2-138.2)</td>
<td>92.4(53.5-153.9)</td>
<td>0.0071</td>
</tr>
<tr>
<td>FEV₁:VC</td>
<td>91.0(75.2-104.5)</td>
<td>98.8(87.3-112.1)</td>
<td>0.0315</td>
</tr>
<tr>
<td>TLC</td>
<td>83.1(73.8-106.5)</td>
<td>102.5(85.5-125.3)</td>
<td>0.0024</td>
</tr>
<tr>
<td>RV</td>
<td>85.1(31.8-125.3)</td>
<td>93.0(58.11-155.3)</td>
<td>0.1461</td>
</tr>
<tr>
<td>DₐCO</td>
<td>88.5(65.0-122.0)</td>
<td>87.1(66.1-127.7)</td>
<td>0.5841</td>
</tr>
<tr>
<td>KCO</td>
<td>97.4(70.0-132.0)</td>
<td>86.1(69.0-115.1)</td>
<td>0.0114</td>
</tr>
<tr>
<td>SpO₂* (%)</td>
<td>95(89-99)</td>
<td>98(96-99)</td>
<td>0.0023</td>
</tr>
<tr>
<td>DₐNO* (ml/min/mHg)</td>
<td>62.7(37.9-83.8)</td>
<td>85.3(44.3-113.3)</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

Table 7-2  Lung function results by SCD status.

Results are presented as median (range) and expressed as a percentage of the predicted value unless indicated*  †Mann-Whitney U test.
Figure 7-1 Impulse oscillometry and spirometry in SCD and control children.

Data are presented as percentage of predicted for height and sex. The red point indicates the median value.
Figure 7-2 Lung volumes and gas transfer in SCD and control children. Data are presented as percentage of predicted for height and sex except where indicated. The red point indicates the median value.
<table>
<thead>
<tr>
<th></th>
<th>SCD children</th>
<th>Controls</th>
<th>p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>18</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Fe\textsubscript{NO,50ml/s}</td>
<td>14.7 (1.6-92.3)</td>
<td>14.6 (1.2-56.1)</td>
<td>0.7569</td>
</tr>
<tr>
<td>J′\textsubscript{aw,NO}</td>
<td>33.5 (6.1-403.6)</td>
<td>60.8 (1.4-319.1)</td>
<td>0.3038</td>
</tr>
<tr>
<td>C\textsubscript{A,NO}</td>
<td>7.0 (1.3-7.3)</td>
<td>2.5 (0.6-5.9)</td>
<td>0.0007</td>
</tr>
<tr>
<td>V′\textsubscript{A,NO}</td>
<td>319.0 (57.3-663.7)</td>
<td>151.0 (31.8-447.1)</td>
<td>0.0224</td>
</tr>
<tr>
<td>Q\textsubscript{pulm}</td>
<td>7.80 (4.25-10.85)</td>
<td>5.50 (3.55-7.70)</td>
<td>0.0006</td>
</tr>
<tr>
<td>Q\textsubscript{I,pulm}</td>
<td>4.97 (3.77-6.82)</td>
<td>3.29 (2.48-4.50)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 7-3 Exhaled NO and pulmonary haemodynamic measurements by SCD status.

†Mann-Whitney U test.
Figure 7-3 Exhaled nitric oxide measurements in SCD and control children.

The red point indicates the median value.
Figure 7-4 Pulmonary blood flow measurements in SCD children and controls.

The red point indicates the median value.
7.3.3 Relationships between lung function, exhaled nitric oxide and pulmonary haemodynamics

In the SCD children and the control group, no significant correlations were observed between exhaled NO indices and Rrs5 or FEV1:VC (table 7.4). In the SCD group, \( Q_{\text{pulm}} \) was positively correlated with \( F_{\text{eNO,50ml/s}} \) (\( p = 0.036 \)), \( C_{\text{A,NO}} \) (\( p = 0.0343 \)) and \( V'_{\text{A,NO}} \) (\( p = 0.0002 \)) (Table 7.5). \( QI_{\text{pulm}} \) was positively correlated with \( F_{\text{eNO}} \) (\( r = 0.591, p = 0.0112 \)), \( C_{\text{A,NO}} \) (\( r = 0.742, p = 0.0006 \)), and \( V'_{\text{A,NO}} \) (\( r = 0.806, p < 0.0001 \)) (Figure 1). No significant correlations were seen between exhaled NO indices and \( Q_{\text{pulm}} \) or \( QI_{\text{pulm}} \) in the control group (table 4) (Figure 7.1).
<table>
<thead>
<tr>
<th>Correlated variables</th>
<th>r†</th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe\textsubscript{NO,50 l/s}</td>
<td>FEV\textsubscript{1}:VC</td>
<td>-0.340</td>
</tr>
<tr>
<td>Fe\textsubscript{NO,50 l/s}</td>
<td>Rrs\textsubscript{5}</td>
<td>0.286</td>
</tr>
<tr>
<td>J′\textsubscript{aw,NO}</td>
<td>FEV\textsubscript{1}:VC</td>
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<tr>
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<td>FEV\textsubscript{1}:VC</td>
<td>-0.179</td>
</tr>
<tr>
<td>C\textsubscript{A,NO}</td>
<td>Rrs\textsubscript{5}</td>
<td>-0.214</td>
</tr>
</tbody>
</table>

| Controls |
|----------------------|-----|-----|
| Fe\textsubscript{NO,50 l/s} | FEV\textsubscript{1}:VC | -0.311 | 0.2234 |
| Fe\textsubscript{NO,50 l/s} | Rrs\textsubscript{5} | -0.245 | 0.3417 |
| J′\textsubscript{aw,NO} | FEV\textsubscript{1}:VC | -0.220 | 0.3805 |
| J′\textsubscript{aw,NO} | Rrs\textsubscript{5} | -0.251 | 0.3153 |
| C\textsubscript{A,NO} | FEV\textsubscript{1}:VC | -0.094 | 0.7108 |
| C\textsubscript{A,NO} | Rrs\textsubscript{5} | 0.267 | 0.2834 |

Table 7-4 Correlations between exhaled NO and markers of airflow obstruction.
†Spearman’s rank correlation.
### SCD children

<table>
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<tr>
<th>Correlated variables</th>
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<th>P&lt;sup&gt;†&lt;/sup&gt;</th>
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<td>V'&lt;sub&gt;A,NO&lt;/sub&gt;</td>
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### Controls

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<th>P&lt;sup&gt;†&lt;/sup&gt;</th>
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</tr>
</tbody>
</table>

Table 7-5 Correlations between exhaled NO and pulmonary haemodynamic measurements.

<sup>†</sup>Spearman’s rank correlation.
Figure 7-5  Alveolar nitric oxide production and pulmonary blood flow in SCD (blue points) and control subjects (red points).

Lines represent the least-square linear regression best fit in each group, the shaded areas are the 95% confidence interval for the fit.
7.4 Discussion

This study has demonstrated that children with SCD compared to controls had an elevated alveolar nitric oxide concentration and increased alveolar production, but their maximal airway nitric oxide flux did not differ from that of ethnic-matched controls. Alveolar production and NO concentration were strongly correlated with pulmonary blood flow in the SCD group but not in the controls, suggesting a link between pulmonary nitric oxide production and a hyper-dynamic circulation. These results differ from those of Radhakrishnan et al [214] who found that airway NO flux but not alveolar NO concentration nor exhaled NO at a flow of 50 ml/s was elevated in SCD. Those differences may be due to a number of factors. All the controls and SCD patients in this study were of African or Caribbean ethnic origin, whereas Radhakrishnan et al recruited controls of various ethnicities, the majority of whom were Caucasian. Furthermore, that study [214] excluded all patients with evidence of airflow obstruction to ensure that patients with asthma and likely elevated $f_{eNO}$ did not influence their results. The work presented earlier in this thesis has, however, suggested that airflow obstruction seen in SCD at least in certain patients may reflect increased pulmonary capillary blood volume rather than asthma [208], hence exclusion of such patients might bias the study population. In adults with SCD Girgis et al [121] reported a reduction in $f_{eNO 50ml/s}$ but used a predominantly Caucasian control group. A larger, more recently reported study by Chaudry et al [204] reported, as in this study, no difference in $f_{eNO 50ml/s}$ between children with SCD and ethnic matched controls. Furthermore,
that study also reported that whilst airflow obstruction, as evidenced by a reduced FEV:VC, was present this was not associated with increased methacholine sensitivity or elevated $f_{e,NO}$. To the best of our knowledge, this study is the first to assess the relationship between pulmonary haemodynamics, exhaled nitric oxide, maximal airway NO flux and alveolar NO concentrations (to estimate alveolar NO production) in children with SCD. The findings that alveolar NO production and concentration were strongly correlated with pulmonary blood flow indices and alveolar NO production was elevated in the SCD group, despite a reduced $D_{l,NO}$, suggest that the altered pulmonary NO dynamics were associated with a hyperdynamic circulation in SCD patients. $F_{eNO, 50ml/s}$ was also correlated with pulmonary blood flow, suggesting that the utility of the standard $F_{eNO, 50ml/s}$ as a marker of airway inflammation may be limited in SCD due to potential contamination of NO from the alveolar region.

In the study by Degano et al of patients with liver cirrhosis, including patients with hepatopulmonary syndrome, alveolar NO concentration and the rate of alveolar NO production were elevated compared to healthy controls [218] and positively correlated with cardiac index (cardiac output normalised for body surface area) measured during right-heart catheterisation. Exhaled NO is also elevated in patients with an ASD [122]. The elevated levels may be due to alveolar nitric oxide production, as the pulmonary haemodynamics were similar to those observed in the SCD patients in this study and the cardiac index was similar to the $Q_{I, pulm}$. The authors of that study [122] report that the role of shear stress may be central as pulmonary blood flow, exhaled NO concentration and plasma NO levels dropped
significantly in children and adults after surgical repair of the ASD. Although a different method for NO measurement was used, the exhaled NO concentrations before ASD closure were similar to the alveolar NO production levels observed in this study. It is reasonable, therefore, to suggest that alveolar nitric oxide concentrations are elevated in children with SCD due to a shear stress and results from an increase in alveolar nitric oxide production. This increase, however, may also be due, at least in part to a greater perfused surface area rather than direct up-regulation of NO production in endothelial cells. The increased pulmonary production of NO occurs despite the functional deficiency of NO which is thought to be a feature of systemic vascular function in SCD disease [58].

This study has strengths and some limitations. A 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. DlNO was also measured and it was therefore possible to demonstrate increased alveolar NO production. Ethnic-specific reference values were used for spirometric results, but static lung volume and impulse oscillometry results were related to reference ranges derived from Caucasian subjects. The two groups, however, 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 study population were African or Caribbean, thereby avoiding confounding from ethnic differences in exhaled NO [215-217]. A non-invasive method was used to measure pulmonary blood flow rather than pulmonary artery catheterisation, but the method and device used has been previously validated in children [174]. Plasma NO products were not measured as
this would have required additional venipuncture, however this would potentially have yielded additional information on overall NO production.

7.5 Conclusion

In conclusion, this study has demonstrated that pulmonary NO production in the alveolar compartment is elevated and correlated with pulmonary blood flow in children with SCD. This is likely to be the result of increased shear stress due to hyper-dynamic circulation. Airway NO flux did not differ from that in healthy ethnic-matched controls and was not related to the airflow obstruction demonstrated in the SCD children. Those results further suggest that airway obstruction at least in certain SCD children is not due to asthma.
8. LONGITUDINAL ASSESSMENT OF LUNG FUNCTION IN CHILDREN WITH SICKLE CELL DISEASE

Note: Baseline lung function tests on Cohort Two were performed by Dr. Karl Sylvester. All other lung function tests and statistical analyses were performed by the candidate.

8.1 Introduction

Lung function abnormalities have been reported even in young SCD children with SCD. One cross-sectional study suggested the occurrence of restrictive abnormalities may increase with increasing age in childhood [138]. More recently, longitudinal studies have been undertaken. In a cohort of 45 children aged between 5 and 18 years measured at baseline and approximately four years later, a predominantly obstructive pattern was reported which increased in prevalence over time; the occurrence of restrictive abnormalities also increased, but to a lesser extent [145]. In contrast, retrospective analysis of a larger cohort of 413 SCD children aged between eight and eighteen years, who were measured on two separate occasions with a variable time to follow-up, demonstrated an increased prevalence of restrictive abnormalities with increasing age [148]. There was a decline in total lung capacity (TLC) of 2.3% per year [148] and a similar decline in forced expiratory volume in one second (FEV1), vital capacity (VC), and forced expiratory flow between 25% and 75% of VC (FEF_{25-75%}). Neither study [145, 148], however, included a control group and thus it is not possible to determine whether
the decline in lung function reported was only seen in SCD patients and hence the magnitude of the effect of SCD. In addition, it is important to assess whether any difference increased with increasing age.

In adults, acute chest syndrome (ACS) is the commonest cause of death [30, 78] and is associated with more severe restrictive disease [222]. In children, ACS episodes have been associated with reductions in lung function [88, 89]. The impact of ACS on longitudinal changes in lung function, however, has not been investigated [145, 148]. There is evidence that asthma may be a risk factor for ACS episodes [83, 86, 88-92]. Hence, in any subsequent longitudinal study the impact of asthma also needs to be considered.

The aim of this prospective study was to examine the rate of decline in lung function in children with SCD in comparison to age and ethnic matched controls. Two cohorts were studied. Cohort one were younger children who were followed for approximately two years, during an age when ACS episodes are more common [80]. Cohort two were older and followed for approximately ten years to allow the long-term changes in lung function during the transition to adulthood to be assessed. Two cohorts were included as comparison of their results would allow the primary hypothesis that the rate of decline in lung function would be greater in the younger cohort as it would relate to their higher occurrence of ACS episodes to be tested. Furthermore, in cohort two, the hypothesis that any obstructive
abnormalities demonstrated at the initial assessment would be replaced by restrictive abnormalities at the subsequent assessment was also tested. As asthma may be a risk factor for ACS episodes [80, 83, 86, 88-92] the study also set out to determine whether asthma and hence baseline obstructive lung function abnormalities influenced any change in lung function and whether obstructive lung function abnormalities were associated with an increased risk of ACS episodes. Ethnic and age matched controls were recruited for both cohorts to determine whether any decline in lung function was only seen in SCD patients.
8.2 Methods

A prospective longitudinal study of children with SCD (homozygous for sickle cell haemoglobin (HbSS)) of African-Caribbean or West African descent was undertaken. Age and ethnic matched children without HbSS were recruited as controls; they were siblings of the SCD children or recruited from local schools. Controls were recruited such that overall group characteristics were matched (Table 8.2); all control subjects were of a similar ethnic background to the children in the SCD group. Two cohorts were tested, each on two occasions. In cohort one changes in lung function over a median follow-up of approximately two years and in cohort two changes in lung function of children over a median follow up of ten years were determined. Cohort two was recruited earlier during a previous period of research grant funding and both cohorts were followed up during a subsequent period of grant funding. Only patients who successfully performed both spirometry and body plethysmography were included in the analysis. The study was approved by the King’s College Hospital Research Ethics Committee and parents gave informed written consent for their child to take part.

8.2.1 Lung function assessments

FEV₁, vital capacity, total lung capacity and residual volume were measured as previously described (Section 3). Spirometry was repeated following administration of a bronchodilator (400μg salbutamol via a MDI and spacer: the MDI was
vigorously shaken between puffs and the delivery of each puff was followed by five normal breaths) and a positive response was defined as an increase in FEV$_1$ of greater than or equal to 12% from baseline. Results were expressed as percent predicted for height, age, and sex using the ethnic-specific reference equations for spirometry [186] and the European Community for Steel and Coal Statement of the European Respiratory Society reference equations for lung volumes and gas transfer [187] for patients over eighteen years of age and those of Rosenthal et al for children under eighteen [202]. The predicted values for total lung capacity were reduced by 12% and residual volume by 7% to correct for ethnicity [136]. Patients were diagnosed with a restrictive abnormality if their TLC was less than the lower limit of normal (LLN) with a normal FEV$_1$:VC, an obstructive abnormality if their FEV$_1$:VC was less than LLN and a mixed pattern if both TLC and FEV$_1$:VC were less than the LLN [136].

8.2.2 Clinical and haematological data

The medical records for all SCD children were examined. ACS was documented by a review of all medical record entries during the follow-up period. All SCD patients who completed two visits were managed clinically at King’s College Hospital and complete records were available for the follow-up period. An ACS episode was diagnosed if the child had suffered chest pain, dyspnoea and pyrexia together with a new pulmonary infiltrate on chest radiograph [78]. SCD children and controls were diagnosed as having asthma if they were currently prescribed any anti-asthma
Prescriptions were issued based on diagnoses made by multiple clinicians. Whether the child was on a chronic transfusion program or receiving hydroxyurea was also noted. Haemoglobin concentrations for the SCD children were obtained from routine clinical blood samples taken within two months of lung function testing and when the patient was clinically stable.

8.2.3 Statistical analysis

Differences in lung function results at baseline and follow-up were assessed for statistical significance using the Wilcoxon signed-rank test, Fisher’s exact test or Chi-squared test as appropriate. Individual lung function results were reported as the percentage predicted in order to normalise results for stature and where appropriate age. Linear mixed model (LMM) analysis was then used to analyse trajectories of decline in lung function in the SCD children relative to the control groups and to determine the influence of asthma, airways obstruction at baseline and the occurrence of ACS during the follow up period. ACS, airways obstruction at baseline and diagnosis of asthma were coded as nominal variables (0 = absent; 1 = present). Models were also fitted to compare the trajectories in the two cohorts. LMM analysis is an extension of multiple regression, which allows irregularly spaced serial data for individuals to be combined in a single linear regression model, providing estimates of both individual changes over time and group patterns. SPPS version 21 (IBM Corp. Armonk, NY), was used to derive all LMMs. In the context of mixed-effect modelling, a fixed factor is defined as a categorical or classification variable, for which the investigator has included all levels (or
conditions) that are of interest in the study. Levels of a fixed factor are chosen so that they represent specific conditions, and can therefore be used to define contrasts of interest in the research study. Fixed effects, also referred to as regression coefficients or fixed-effect parameters, describe the relationship between the dependent variable and predictor variables (i.e., fixed factors or continuous covariates) for an entire population of units of analysis, or for a limited number of subpopulations defined by the levels of a fixed factor. Fixed effects may describe contrasts or differences between levels of a fixed factor (e.g. between patients with and without a history of ACS) in terms of mean responses for the continuous dependent variable, or they may describe the effect of a continuous covariate on the dependent variable. Fixed effects are assumed to be unknown fixed quantities in an LMM (directly equivalent to the coefficients in a standard linear regression), and are estimated based on the analysis of the data collected in a given research study.

Conversely, a ‘random factor’ is defined as a classification variable with levels that can be thought of as being randomly sampled from a population of levels being studied. All possible levels of the random factor are not present in the data set, but it is the aim of the analysis to make inferences about the entire population of levels. The term ‘random effects’, therefore, does not refer to the temporal or spatial distribution of the model variables, but to the fact that these values represent random deviations from the relationships described by the fixed effects. Specifically, random effects associated with the levels of a random factor can enter an LMM as random intercepts (representing random deviations for a given subject
from the overall fixed intercept), or as random coefficients (representing random deviations for a given subject from the overall fixed slope). For a full technical discussion, see West [223] and West & Galecki [224]. Fixed effects in the models were: ACS during the study period, diagnosis of asthma, obstruction at baseline, age at measurement, and an intercept term. In the models comparing SCD subjects with controls, and the two cohorts, SCD status or cohort membership, respectively, were entered as fixed effects. Random effect variables comprised age at measurement and an intercept term in addition to the subject study ID, to characterise the subject-level deviation from the estimated fixed effects. A first-order autoregressive covariance structure was used for random effects. Models were estimated using restricted maximum likelihood (REML), and separate models were fitted for each of the lung function results with all models grand-mean centred on age at baseline such that the intercept terms would be interpretable as the mean value of the result at baseline, and the slope would represent the annual rate of change. Interaction terms were introduced to test the equality of slopes between groups. Initial models included all variables and interactions. The validity of including the random effects, and the selection of an appropriate covariance structure for the residuals was assessed by likelihood ratio testing based on the $-2$ REML log-likelihood difference between the original and modified models. Models were then reduced by the sequential removal of non-significant terms. Likelihood ratio tests based on the difference in $-2$ ML log-likelihood for initial and reduced models were used to determine whether a non-significant term should be removed [223]. Baseline predictors of future ACS were determined using binary logistic regression.
8.3 Results

8.3.1 Subjects

**Cohort 1:** Forty-seven children with SCD and twenty-six controls were assessed; the two groups were of similar age and sex distribution (Table 8.1). Ten SCD children (21%) had had at least one ACS episode during the study. Nine SCD children and two controls had a diagnosis of asthma (p = 0.308). Seven SCD children (14.9%) were prescribed hydroxyurea and one (2.1%) was on a chronic transfusion programme within the study period. The majority of the SCD children patients (45) and controls (24) were aged five or above at baseline (96% and 92%, respectively).

**Cohort 2:** Forty-five children with SCD and twenty-six controls were assessed; the two groups were of similar age and sex distribution (Table 8.1). Twelve SCD children (27%) had had at least one ACS episode during the study. Three SCD children and four controls had a diagnosis of asthma (p = 0.227). Eight SCD children (17.7%) were prescribed hydroxyurea and two (4.4%) was on a chronic transfusion programme within the study period. The majority of SCD children (43) and controls (24) were aged five or above (96% and 96%, respectively).

Cohort one was significantly younger at baseline compared to cohort two (p = 0.007) (Table 8.1). The only significant difference in lung function of the two SCD
cohorts at recruitment was that cohort one had a higher TLC (p=0.0247). The children in Cohort One had a mean of 0.58 (range 0–8.7) ACS episodes/year and Cohort Two a mean of 0.09 (range 0–1.3) ACS episodes (p=0.0355). If only the children who had at least one ACS episode were considered, in Cohort One this was a median of 0.65 (range 0.38–8.7) episodes/year and in Cohort Two 0.11 (range 0.08–1.26) episodes/year (p<0.0001). There were no significant differences in the lung function results of the two control groups at recruitment.
<table>
<thead>
<tr>
<th></th>
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<th>Controls</th>
<th>p</th>
</tr>
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<td></td>
<td></td>
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</tr>
<tr>
<td>n</td>
<td>47</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Males (%)</td>
<td>21 (45%)</td>
<td>7 (27%)</td>
<td>0.352‡</td>
</tr>
<tr>
<td>Age at baseline (years)</td>
<td>8.8 (3.0-13.1)</td>
<td>10.2 (4.0-14.6)</td>
<td>0.072†</td>
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<td>Time to follow-up (years)</td>
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<td>1.7 (1.5-2.51)</td>
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<td>Hb (g/dl)</td>
<td>8.2 (4.6-11.9)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Cohort 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>45</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Males (%)</td>
<td>19 (42.2%)</td>
<td>8 (33.3%)</td>
<td>0.81‡</td>
</tr>
<tr>
<td>Age at baseline (years)</td>
<td>10.2 (4.3-16.0)</td>
<td>8.5 (4.0-17.8)</td>
<td>0.24†</td>
</tr>
<tr>
<td>Time to follow-up (years)</td>
<td>9.9 (6.0-13.5)</td>
<td>11.4 (7.0-13.4)</td>
<td>0.063†</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>8.2 (5.7-12.8)</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Table 8-1 Demographics by SCD status.

Data are given as n (%) or median (range). Mann-Whitney U test. ‡Fisher’s exact test.
8.3.2 Lung function changes with increasing age

In both cohorts, lung function declined significantly in the SCD children, but in neither control group (Tables 8.2 and 8.3, Figure 8.1).

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<th>Baseline n=47</th>
<th>Follow-up n=47</th>
<th>p†</th>
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<td>FEV(_1)</td>
<td>91.6 (70.5-117.5)</td>
<td>88.7 (55.5-122.6)</td>
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<tr>
<td>VC</td>
<td>97.2 (67.3-140.8)</td>
<td>91.8 (59.4-123.5)</td>
<td>0.0002</td>
</tr>
<tr>
<td>FE_F(_{25-75})</td>
<td>91.8 (40.4-189.6)</td>
<td>82.4 (29.7-146.7)</td>
<td>0.0001</td>
</tr>
<tr>
<td>FEV(_1):VC</td>
<td>94.7 (71.8-109.8)</td>
<td>95 (72.2-111.8)</td>
<td>0.2318</td>
</tr>
<tr>
<td>TLC</td>
<td>97.2 (72.1-127.1)</td>
<td>89.7 (68.5-121.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RV</td>
<td>107.4 (40.6-212.0)</td>
<td>95.5 (42.2-160.0)</td>
<td>0.0032</td>
</tr>
<tr>
<td>RV/TLC</td>
<td>128.2 (68.2-218.5)</td>
<td>120.1 (52.5-182.8)</td>
<td>0.469</td>
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<table>
<thead>
<tr>
<th>Controls n=26</th>
<th>n=26</th>
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</thead>
<tbody>
<tr>
<td>FEV(_1)</td>
<td>94.2 (73.4-129.3)</td>
</tr>
<tr>
<td>VC</td>
<td>98.9 (79.6 – 129.2)</td>
</tr>
<tr>
<td>FE_F(_{25-75})</td>
<td>96.3 (37.5-160.1)</td>
</tr>
<tr>
<td>FEV(_1):VC</td>
<td>98.5 82.3-108.8)</td>
</tr>
<tr>
<td>TLC</td>
<td>94.8 (80.8-115.5)</td>
</tr>
<tr>
<td>RV</td>
<td>101.4 (66.6-172.2)</td>
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<td>RV/TLC</td>
<td>104.7 (72.1-137.1)</td>
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</table>

Table 8-2 Lung function in cohort one by follow up status.

Data are presented as expressed as percent predicted and median (range). †Wilcoxon signed-rank test.
<table>
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<th>Follow-up n=45</th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV₁</td>
<td>90.7 (64.0-117.2)</td>
<td>81.2 (66.4-106.7)</td>
<td>0.0002</td>
</tr>
<tr>
<td>VC</td>
<td>97.6 (62.6-116.7)</td>
<td>85.4 (68.7-109.6)</td>
<td>0.0003</td>
</tr>
<tr>
<td>FEF₂₅-₇₅</td>
<td>91.8 (45.9-144.9)</td>
<td>74.5 (28.9-122.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FEV₁:VC</td>
<td>96.2 (69.7-109.4)</td>
<td>95.4 (64.4-108.1)</td>
<td>0.7648</td>
</tr>
<tr>
<td>TLC</td>
<td>92.5 (67.6-127.1)</td>
<td>81.6 (61.0-108.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RV</td>
<td>101.2 (37.7-212.0)</td>
<td>88.9 (54.8-149.2)</td>
<td>0.0300</td>
</tr>
<tr>
<td>RV/TLC</td>
<td>121.2 (73.4-194.3)</td>
<td>113.2 (67.5-221.5)</td>
<td>0.0692</td>
</tr>
<tr>
<td>Controls n=24</td>
<td>n=24</td>
<td>n=24</td>
<td>n=24</td>
</tr>
<tr>
<td>FEV₁</td>
<td>99.8 (73.8-128.8)</td>
<td>100.3 (76.8-148.7)</td>
<td>0.0946</td>
</tr>
<tr>
<td>VC</td>
<td>103.1 (71.3-131.2)</td>
<td>102.7 (86.2-124.6)</td>
<td>0.1747</td>
</tr>
<tr>
<td>FEF₂₅-₇₅</td>
<td>92.7 (55.3-168.3)</td>
<td>102.3 (54.7-163.0)</td>
<td>0.0891</td>
</tr>
<tr>
<td>FEV₁:VC</td>
<td>98.8 (81.1-112.2)</td>
<td>96.3 (84.9-116.6)</td>
<td>0.7425</td>
</tr>
<tr>
<td>TLC</td>
<td>98.3 (78.5-111.8)</td>
<td>94.0 (84.2-121.8)</td>
<td>0.9421</td>
</tr>
<tr>
<td>RV</td>
<td>95.7 (50.5-165.1)</td>
<td>91.1 (33.0-154.3)</td>
<td>0.9431</td>
</tr>
<tr>
<td>RV/TLC</td>
<td>110.2 (63.2-175.7)</td>
<td>94.6 (45.0-158.7)</td>
<td>0.0742</td>
</tr>
</tbody>
</table>

Table 8-3 Lung function in cohort two by follow up status.

Data are presented as expressed as percent predicted and median (range). †Wilcoxon signed-rank test.
Figure 8-1 Trajectories over time for FEV₁ and FEF₂₅/₇₅ (shown as z-scores) in SCD and control groups. Individual data are shown by linked data points.
8.3.3 Lung function abnormalities

At baseline, sixteen SCD children in cohort one (34%) had an obstructive pattern, and one (2%) a restrictive defect; no child had a mixed defect. At follow-up, the number of subjects with an obstructive defect had declined to five (10.6%, \( p = 0.021 \)), the number with restrictive defects increased to eight (17%, \( p = 0.034 \)) and two patients had a mixed pattern (4.3%, \( p = 0.495 \)). A positive bronchodilator was seen in four SCD children (16.7%) at baseline and three (12.5%) at follow-up (\( p = 1.000 \)). In cohort two, at baseline, eleven SCD children (24%) had an obstructive pattern and five (11%) a restrictive defect; no child had a mixed defect. At follow-up, five subjects had an obstructive defect (11.1%, \( p = 0.167 \)), the number with restrictive defects had increased to twenty (44%, \( p = 0.0008 \)) and three patients had a mixed pattern (6.7%, \( p = 0.242 \)). A positive bronchodilator response was seen in seven subjects (15.6%) at baseline and one subject (2.2%) at follow-up (\( p = 0.059 \)).

At baseline, the proportion of SCD children with obstructive and restrictive defects was similar in both cohorts (\( p = 0.364 \), \( p = 0.107 \) respectively). At follow-up, cohort two compared to cohort one had a greater proportion of SCD children with a restrictive defect (\( p = 0.006 \)), but a similar number with an obstructive defect (\( p = 0.792 \)). In both cohorts, there were significant correlations in the SCD children between obstructive defects at baseline and ACS episodes (\( p = 0.0003 \) for cohort one, \( p = 0.028 \) for cohort two). In cohorts one and two, in the SCD children the presence of obstructive defects at baseline was predictive of an ACS episode during
the study: odds ratio (OR) 13.9 (95%CI 2.5 – 77.0), p= 0.003 and 5.4 (95%CI 1.2 – 23.7), p= 0.026 respectively. In cohort one there was a trend in the SCD children for an independent association between asthma and a subsequent ACS episode: OR 6.1 (95% CI -0.5 – 12.8, p = 0.070). ACS episodes occurred more frequently in the SCD children in cohort one than cohort two (one episode per 1.93 patient/years versus one episode per 12.6 patient/years (p<0.0001) (Figure 8.2).
In neither control group was there a change in the patterns of lung function over the follow up period. Amongst the controls in cohort one, three had an obstructive abnormality and none a restrictive abnormality at baseline and follow up. Amongst
the controls in cohort two, two had an obstructive lung function abnormality and none a restrictive abnormality at baseline and follow up.

In cohort 1, a significant decline was observed in the SCD children relative to the controls in FEV₁ (p=0.027), VC (p<0.0001), FEF₂₅₋₇₅ (p=0.042), TLC (p<0.0001) and RV (p=0.001) (Table 8.4). In cohort 2, a significant decline was observed relative to the controls in FEV₁ (p=0.001), VC (p=0.004), FEF₂₅₋₇₅ (p=0.016) and TLC (p=0.002) (Table 8.5). The rate of decline was significantly greater in cohort one than cohort two for FEV₁ (p = 0.008), VC (p = 0.001), FEF₂₅₋₇₅ (p = 0.030), TLC (p = 0.004), and RV (p = 0.043) (Table 8.6).
<table>
<thead>
<tr>
<th></th>
<th>Difference from control</th>
<th>Test of difference from control group (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intercept</td>
<td>Slope</td>
</tr>
<tr>
<td>FEV₁</td>
<td>-7.64</td>
<td>-1.768</td>
</tr>
<tr>
<td>VC</td>
<td>-3.36</td>
<td>-4.062</td>
</tr>
<tr>
<td>FEV₁:VC</td>
<td>-5.10</td>
<td>1.089</td>
</tr>
<tr>
<td>FEF₂₅₋₇₅</td>
<td>-14.7</td>
<td>-1.651</td>
</tr>
<tr>
<td>TLC</td>
<td>0.77</td>
<td>-2.800</td>
</tr>
<tr>
<td>RV</td>
<td>5.80</td>
<td>-3.316</td>
</tr>
<tr>
<td>RV:TLC</td>
<td>5.16</td>
<td>-1.565</td>
</tr>
</tbody>
</table>

Table 8-4 Linear mixed models for cohort one.

Intercepts are in percent predicted, slopes percent predicted per year.
<table>
<thead>
<tr>
<th></th>
<th>Difference from control group</th>
<th>Test of difference from control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intercept</td>
<td>Slope</td>
</tr>
<tr>
<td><strong>FEV\textsubscript{1}</strong></td>
<td>-9.34</td>
<td>-0.908</td>
</tr>
<tr>
<td><strong>VC</strong></td>
<td>2.15</td>
<td>-0.931</td>
</tr>
<tr>
<td><strong>FEV\textsubscript{1}:VC</strong></td>
<td>-3.65</td>
<td>0.166</td>
</tr>
<tr>
<td><strong>FEF\textsubscript{25-75}</strong></td>
<td>-5.22</td>
<td>-1.803</td>
</tr>
<tr>
<td><strong>TLC</strong></td>
<td>-2.88</td>
<td>-0.985</td>
</tr>
<tr>
<td><strong>RV</strong></td>
<td>6.19</td>
<td>-1.059</td>
</tr>
<tr>
<td><strong>RV:TLC</strong></td>
<td>9.58</td>
<td>0.772</td>
</tr>
</tbody>
</table>

Table 8-5 Linear mixed models for cohort two.

Intercepts are in percent predicted, slopes percent predicted per year
Difference from cohort 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intercept</th>
<th>Slope</th>
<th>Intercept</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV₁</td>
<td>-1.56</td>
<td>1.402</td>
<td>0.472</td>
<td>0.008</td>
</tr>
<tr>
<td>VC</td>
<td>-1.00</td>
<td>1.965</td>
<td>0.692</td>
<td>0.001</td>
</tr>
<tr>
<td>FEV₁:VC</td>
<td>0.16</td>
<td>0.014</td>
<td>0.919</td>
<td>0.971</td>
</tr>
<tr>
<td>FEF₂₅-₇₅</td>
<td>5.48</td>
<td>2.253</td>
<td>0.310</td>
<td>0.030</td>
</tr>
<tr>
<td>TLC</td>
<td>-2.80</td>
<td>1.563</td>
<td>0.251</td>
<td>0.004</td>
</tr>
<tr>
<td>RV</td>
<td>-1.88</td>
<td>2.484</td>
<td>0.711</td>
<td>0.043</td>
</tr>
<tr>
<td>RV:TLC</td>
<td>23.8</td>
<td>0.333</td>
<td>&lt;0.0001</td>
<td>0.783</td>
</tr>
</tbody>
</table>

Table 8-6 Linear mixed models for cohort two relative to cohort one.

Intercepts are in percent predicted, slopes percent predicted per year

In cohort two, SCD patients with an obstructive defect at baseline were more likely to have a restrictive abnormality at follow-up (univariate logistic regression, p=0.0289). On LMM analysis on the SCD children in cohort one, obstructive defects at baseline were linked to a lower intercept for FEV₁ (p = 0.027) and FEV₁:VC (p < 0.0001). The occurrence of ACS episodes were associated with a more rapid decline (indicated by a negative coefficient on ACS; table 8.7) greater negative slope in FEV₁ (p=0.005), VC (p=0.022), FEF₂₅-₇₅ (p=0.003) and TLC (p=0.004). In cohort two, obstructive defects at baseline were linked to a lower intercept for FEV₁ (p = 0.011), FEV₁:VC (p < 0.0001), and FEF₂₅-₇₅ (p = 0.013), whilst ACS episodes were associated with a more rapid decline in (indicated by a negative coefficient on ACS; table 8.8) VC (p = 0.031) and TLC (p = 0.026), and an increase in for FEV₁:VC over time (p =
0.049) (Tables 8.7 and 8.8). No significant effects of hydroxyurea use or chronic transfusion were seen during exploratory analysis when those factors were included as covariates in the models, nor did their inclusion as non-significant terms improve the model fit as assessed by likelihood ratio testing.
<table>
<thead>
<tr>
<th>Covariate</th>
<th>Coefficient (95%CI) †</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV₁</td>
<td></td>
<td></td>
</tr>
<tr>
<td>For Intercept</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obstructive</td>
<td>-7.19 (-13.52 to -0.86)</td>
<td>0.027</td>
</tr>
<tr>
<td>For Slope</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACS</td>
<td>-2.21 (-3.70 to -0.72)</td>
<td>0.005</td>
</tr>
<tr>
<td>VC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>For Slope</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACS</td>
<td>-3.04 (-5.61 to -0.46)</td>
<td>0.022</td>
</tr>
<tr>
<td>FEV₁:VC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>For Intercept</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obstructive</td>
<td>-13.15 (-17.00 to -9.30)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>For Slope</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACS</td>
<td>-3.75 (-6.18 to -1.33)</td>
<td>0.003</td>
</tr>
<tr>
<td>TLC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>For Slope</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACS</td>
<td>-3.11 (-5.21 to -1.00)</td>
<td>0.004</td>
</tr>
<tr>
<td>RV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>For Slope</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obstructive</td>
<td>6.61 (4.10 to 9.11)</td>
<td>0.047</td>
</tr>
<tr>
<td>RV:TLC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>For Slope</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obstructive</td>
<td>4.26 (0.06 to 8.460)</td>
<td>0.047</td>
</tr>
</tbody>
</table>

Table 8-7 Final linear mixed models assessing the effect of covariates in cohort one.

The coefficient on slope indicates the additional rate of decline with age when covariate is present; the coefficient on intercept represents the difference in baseline value when covariate is present.

Intercepts are in percent predicted, slopes percent predicted per year.

†Represents mean effect of corresponding binary input variable. Interaction effects between predictors were not considered.
<table>
<thead>
<tr>
<th>Covariate</th>
<th>Coefficient (95%CI)†</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FEV₁</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>For Intercept</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obstructive</td>
<td>-8.78 (-15.45 to -2.11)</td>
<td>0.011</td>
</tr>
<tr>
<td>VC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>For Slope</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACS</td>
<td>-1.03 (-1.97 to -0.95)</td>
<td>0.031</td>
</tr>
<tr>
<td><strong>FEV₁:VC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>For Intercept</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obstructive</td>
<td>-16.65 (-21.46 to -11.83)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>For Slope</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obstructive</td>
<td>1.172 (0.68 to 1.66)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>FEF₂₅-₇₅</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>For Intercept</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obstructive</td>
<td>-13.29</td>
<td>0.013</td>
</tr>
<tr>
<td><strong>TLC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>For Intercept</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACS</td>
<td>-1.08 (-2.04 to -0.13)</td>
<td>0.026</td>
</tr>
<tr>
<td>For Slope</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACS</td>
<td>-1.068</td>
<td>0.030</td>
</tr>
</tbody>
</table>

Table 8-8 Final linear mixed models assessing the effect of covariates in cohort two.

The coefficient on slope indicates the additional rate of decline with age when covariate is present; the coefficient on intercept represents the difference in baseline value when covariate is present. Interaction effects between predictors were not considered.

Intercepts are in percent predicted, slopes percent predicted per year.

†Represents mean effect of corresponding binary input variable.
8.3.4 Exploratory analysis of combined cohorts

Noting the degree of overlap between Cohort One and Cohort Two, an exploratory analysis was performed to determine whether the two cohorts (including SCD and control subjects) could be merged. This would increase statistical power and might demonstrate the age-dependency of the rate of decline of lung function more clearly. Since this would be a departure from the prospective study design, preliminary statistical diagnostics were performed to determine whether it would be appropriate to fit mixed-effect models to the combined data-set. A particular concern was the potential effect of the marked difference in time to follow-up between the two cohorts on the statistical properties of the combined data. To examine this, a probability density plot was produced for the time to follow up using a Gaussian kernel density function [225]; an approximately normal (i.e., unimodal) distribution would suggest that model fitting as a single dataset would be valid. Visual inspection of the plot suggested a strongly bimodal distribution (Figure 8.2). Hartigan’s dip test was then used test the formal hypothesis that the distribution of time to follow-up did not differ significantly from the normal distribution [226]: this confirmed non-unimodality (p<0.0001). To assess the impact on the validity of modelling the combined cohorts, LMMs were derived for FEV$_1$, FEF$_{25/75}$ and TLC. The model structure and covariates were the same described for the separate cohorts in section 8.3.2. Quantile-quantile (Q-Q) plots were obtained to determine if the model residuals for each level of the random effects were normally distributed (Figures 8.3-8.6). For each of the lung function indices, marked nonlinearities were observed on the Q-Q plot, indicating that the
residual distribution was strongly non-normal. Regression model assumptions were, therefore not satisfied and any conclusions drawn from LMMs on the combined data would likely be invalid. Based on the results of the exploratory analysis, it was decided to retain the original analysis scheme and not proceed further with analysis of the combined cohorts.

Figure 8-2  Kernel density plot and histogram showing the probability density function for time to follow-up for Cohort One and Cohort Two combined. A bimodal distribution is evident.
Figure 8-3  Quantile-quantile plot for the standardised residuals of a LMM for FEV\textsubscript{1} in Cohort One and Cohort Two combined.

For a normal distribution, points should lie on a straight line $y = \alpha x$. Deviations from linearity indicate model assumptions have not been met.
Figure 8-4 Quantile-quantile plot for the standardised residuals of a LMM for FEF_{25/75} in Cohort One and Cohort Two combined.

For a normal distribution, points should lie on a straight line y=αx. Deviations from linearity indicate model assumptions have not been met.
Figure 8-5 Quantile-quantile plot for the standardised residuals of a LMM for TLC in Cohort One and Cohort Two combined.

For a normal distribution, points should lie on a straight line $y = \alpha x$. Deviations from linearity indicate model assumptions have not been met.

### 8.4 Discussion

This study has demonstrated that lung function declines in children with SCD compared to similar aged, ethnic matched controls. The average annual rate of decline for all lung volumes was significantly greater in cohort one compared to cohort two, which suggests the most rapid period of deterioration takes place during early childhood. These data also demonstrated that ACS episodes were the only independent predictor of a greater decline in lung volumes. ACS episodes occurred more frequently per unit follow up period in cohort one who were followed up for two years compared to cohort two who were followed up for ten
years. The impact of this finding on lung function decline is likely to reflect to the longer time to follow-up in Cohort One compared to Cohort Two. In both cohorts, the frequency of ACS was greatest when patients were younger. In cohort two, therefore, ACS frequency would be dropping for much of the long follow-up period. Given that patients were measured at only two time points, this would tend to reduce the average rate of decline when compared with Cohort One, who were followed for a shorter time when younger. The younger age of Cohort One at baseline is also likely to have boosted the ACS frequency in this group. Probability density plots, indicating the frequency of ACS for the two Cohorts separately, and for Cohort One and Cohort Two combined are shown in Figure 8. These findings suggest a nonlinear trajectory for lung function decline in children SCD: definitive confirmation of this hypothesis would, ideally, entail more frequent measurements over the entire course of childhood and adolescence with testing taking place between each successive episode of ACS. Such a study would, however, be extremely challenging to implement and require substantial resources. Thus ACS episodes might explain the greater rate of decline in lung function in cohort one and hence these results emphasize the need for more effective methods of preventing ACS episodes, such as an increased use of hydroxyurea.

The rate of decline in lung volumes observed in the SCD children in cohort one, that is the younger children, was similar to that demonstrated by Maclean et al [148], who observed an annual reduction in TLC of around 2.3% per year, with proportionate reductions in FEV₁, VC, and FEF₂⁵-₇⁵. Unlike in the study of Maclean
et al, however, a decline in RV:TLC was not seen in the SCD children. This difference may be explained by Maclean not expressing RV:TLC as a percentage of the predicted value and therefore not accounting for age-related changes in RV:TLC [190].

The prevalence of obstructive lung disease in the SCD children at baseline was similar in both cohorts (34 and 24% respectively) and to that observed by Koumbourlis et al (22%) [145], but higher than that reported by MacLean et al. The latter difference may be due to MacLean’s use of a fixed cutoff for FEV$_1$:VC to define an obstructive defect, whereas this study used the lower limit of normal based on a reference range. After criticism from Koumbourlis [227], Maclean and coworkers subsequently reanalyzed their data using the lower limit of normal to define abnormality and found that more patients had a diagnosis of obstruction than reported in the original study [228]. The proportion of patients with restrictive disease at baseline was lower in the SCD children cohort one to that observed by Koumbourlis (22%), but similar in cohort two. Cohort one was, however, younger than the cohort of Koumbourlis (8.8 versus 10.6 years), whereas cohort two was of a similar age (10.2 years) [16]. The prevalence of restrictive defects at baseline was higher at follow-up in cohort two than in Maclean’s cohort when assessed at age seventeen (18.7%) [148]. That difference may be explained by Maclean using a fixed cutoff of <70% predicted for TLC to define restriction, which may have resulted in mis-diagnosis.
Obstructive defects at baseline, but not asthma significantly increased the likelihood of the child suffering an ACS episode. The incidence of obstructive defects at baseline was higher than that of a diagnosis of asthma, which suggests other mechanisms may be responsible for obstructive defects in SCD. Indeed, in the studies described in chapters 4-6 of this thesis, an association between pulmonary vascular engorgement and obstructive lung function in both adults [208] and children with SCD [15] was demonstrated. Hence, any preventative strategy needs to consider this abnormality in SCD children.

This study has strengths and some limitations. A strength was the inclusion of two control groups of similar age, sex and race to the SCD children; to the best of the author’s knowledge, this is the first longitudinal study to include matched controls evaluated in parallel with SCD children. Patients were also monitored prospectively and a consistent definition used to document the occurrence of ACS episodes during the study period. The inclusion of two SCD groups with two and ten-year follow-up periods respectively allowed both the short and long term changes in lung function in childhood to be evaluated. Ethnic-specific reference equations were used for spirometric results, but the static lung volume results were related to two reference ranges derived from Caucasian children and adults with a fixed correction factor to account for ethnicity. The change in lung function was, therefore, referenced to an ethnic-matched control group in each cohort so that interpretation of within-cohort changes in the SCD children was valid. Indeed, the pattern of change in static lung volumes was similar to that in spirometric
measurements. Patients who were treated with hydroxyurea or chronic transfusions were not excluded, as this would have biased the study population in favor of SCD children with less severe disease. Inclusion of those treatments into the analysis did not result in any significant differences in the results, but the number of children receiving such treatments was small and hence it would be inappropriate to draw conclusions from those results. SPSS version 21 was used for mixed-effect modelling: SPSS uses the Satterthwaite approximation of Fai and Cornelius [229] to calculate degrees of freedom for testing fixed effects; this method can result in negatively biased model-based standard errors, particularly where sample-sizes are small, compared to the Kenward-Roger [230] method implemented by the SAS and R statistics packages, which may result in a greater degree of model uncertainty. However, the internal consistency of the models across lung function indices suggests that this potential limitation did not substantially hamper interpretability. Additionally, the final models for FEV$_1$ and FEF$_{25/75}$ given in Tables 8.4 and 8.5 were re-fitted using the ‘R’ statistical suite (package ‘lme4’), and the ‘lmerTest’ package was used to compare model F-statistics generated by the Satterthwaite and Kenward-Roger procedures [231]. No substantial difference was noted, further suggesting that this limitation did not adversely impact model validity.
8.5 Conclusions

In conclusion, lung function in children with sickle cell disease declines and the rate of decline was greater in young children in whom ACS episodes were more common. These results suggest treatment strategies to prevent ACS episodes need to be started in young SCD children if they are to be most effective in preventing the decline in lung function.
9. GENERAL DISCUSSION AND CONCLUSIONS

9.1 Lung function and pulmonary vascular abnormalities

The data presented in chapter four and five of this thesis demonstrate an association between changes in pulmonary vascular morphology consistent with increased vascular volume and lung function abnormalities in adults and children with SCD. In a cohort of adults with HbSS disease, two measures of small-vessel size were derived from CT scans. The segmental A/B ratio and CSA<5 mm% (which provides a measure of the total cross-sectional area of pulmonary arteries and veins at the sub-subsegmental level) were both independently linked to reductions in FEV₁, VC and FEF₂₅/₇₅ and to increased respiratory system resistance and RV:TLC. These results are consistent with those from the cohort of children assessed in chapter five, in whom pulmonary capillary blood volume was negatively correlated with FEV₁, VC and FEF₂₅-₇₅ and positively correlated with respiratory system resistance. The negative relationship between measures of pulmonary vascular volume and VC, FEV₁ and FEF₂₅/₇₅ and a positive relationship with RV, RV:TLC and respiratory system resistance suggest that vascular dimensions are related to an obstructive defect. It should be noted, however, that VC, FEV and FEF₂₅/₇₅ can be reduced in both obstruction and restriction and thus the correlations with markers of vascular dilation and reductions in VC, FEV₁ and FEF₂₅-₇₅ may also indicate a relationship between vascular dilation and the development of restrictive lung
function. No relationships were observed, however, between vascular volume and TLC. Additionally, measures of pulmonary vascular volume correlated with reduced haemoglobin concentration in both studies, suggesting that haemolysis and chronic anaemia, rather than vaso occlusion, is the key process driving these pulmonary function abnormalities.

In chapter six, evidence for a causal link between increased pulmonary capillary blood volume and airflow obstruction was acquired. Significant increases in pulmonary capillary blood volume and airways resistance were seen to occur immediately following blood transfusion in children with SCD. Those changes were accompanied by significant reductions in FEV₁, VC and FEF₂₅₋₇₅ and the change in lung function correlated with the increase in pulmonary capillary blood volume.

Chapter seven presented evidence that a hyper-dynamic pulmonary circulation also impacts on pulmonary nitric oxide dynamics. Elevated alveolar pulmonary NO production significantly correlated with pulmonary blood flow which may reflect the increased shear stress due to a hyper-dynamic circulation. Airway NO flux was not elevated in the SCD children compared to the controls nor did it correlate with indices of airways obstruction. The data from these four studies therefore suggest that airways obstruction in at least some SCD patients is not due to asthma.
9.1.1 Interpretation of the results and speculation on possible mechanisms

There are a number of pathways by means of which an engorged pulmonary circulation might impact on lung function.

1. Compression via peribronchial sheaths

The peripheral airways lie within bronchovascular sheaths which are embedded in the lung parenchyma. These sheaths also enclose branches of the pulmonary vascular tree as well as lymphatic vessels, and the close anatomical proximity of the pulmonary vessels may allow engorged pulmonary vessels to directly compress the distal airways [232-234]. Studies in dogs with experimental pulmonary oedema have demonstrated an increase in peripheral airways resistance [233]. Morphological analysis of these animals revealed that competition for space between pulmonary arteries/arterioles and small airways in the bronchovascular sheaths explained the increase in peripheral airways resistance observed when congestion was induced by elevating left atrial pressure. Furthermore, the peripheral airflow obstruction was reversible when congestion was relieved [233], provided that left atrial pressure did not exceed approximately 15mmHg. At higher pressures, the peripheral obstruction was irreversible, and was attributed to alveolar and interstitial oedema. It is also possible that engorged lymphatic vessels may impact on distal airways in a similar manner. Interestingly, as described in chapter four, subpleural curvilinear bands were amongst the commonest parenchymal abnormalities observed in the cohort of adult SCD patients. This
unusual sign was noted on CT in 26% of SCD patients. Subpleural curvilinear bands have also been observed in subjects without lung disease undergoing lymphography and this has led to the suggestion that the sign may represent an engorged subpleural lymphatic network [195]. In patients with SCD this may occur as a result of interstitial oedema and increased lymphatic drainage.

2. **Bronchial vessel dilatation**

Interaction between with the pulmonary and bronchial circulation may also be involved in vascular-mediated airways disease. The bronchial circulation serves primarily to supply nutrients to the bronchial and parenchymal structures of the lung [235, 236], including the tissues and bronchial mucosa of the large and small airways [237]. The bronchial mucosa is heavily vascularised with a dense network of interconnected vascular networks laid around and within each bronchiole. Importantly, the mucosal vascular plexus is situated close to the airway epithelial surface and it has been estimated that the majority of bronchial mucosal capillaries are located within 100 micrometres of the airway lumen; engorgement of these vessels may, therefore, result in airway oedema [238-240]. This may be relevant in situations where pulmonary vascular engorgement or vasculopathy are known to occur, since the bronchial vasculature has distinct flow characteristics which may enable interaction with the pulmonary venous circulation. In humans, the blood supply for the bronchial circulation originates in the systemic vascular system, primarily from branches of the descending aorta with successive divisions following
distally with increasing generations of the airway tree. Crucially, a large fraction of
the post-capillary bronchial vessels anastomose with the venous pulmonary
circulation and approximately 65% or more of the total flow through the bronchial
circulation returns to the left heart through these anastomoses [241-243]. This
means that flow through the bronchial vasculature may be influenced by
pulmonary vascular pressures, particularly pulmonary venous pressure. Indeed, as
Lockhart *et al* have shown, any increase in the outflow pressure of the bronchial
circulation, which is close to left atrial pressure, will facilitate microvascular leakage
and hence airway oedema [244]. This is intriguing in light of one of the findings in
adults with SCD presented in chapter four. Here, a positive correlation was
observed between CSA<5mm% (a CT measure of the aggregate cross-sectional area
of the distal arteries and veins) and the echocardiographic E/e’ which is a marker of
left atrial filling pressure. A higher E/e’ would result in some elevation of pulmonary
venous pressure, and since the CSA<5mm% was the strongest predictor of impaired
lung function, it is tempting to speculate that interaction between a hyperdynamic
pulmonary circulation and the bronchial vasculature may have a role in the
development of the obstructive lung function abnormalities observed.

A further mechanism by which the bronchial circulation might become engorged
and thereby impinge on the airways arises from its ability to undergo expansion in
the event of a compromised pulmonary circulation [245]. This is thought to occur
as a compensatory mechanism, as the engorged bronchial circulation is able to
contribute substantially to gas exchange function [235]. In chronic lung disease
where regional pulmonary perfusion is reduced the increase in bronchial blood
flow may be massive. For example, in patients with chronic thromboembolic pulmonary hypertension (CTPH), the bronchial circulation has been shown to expand to such a degree that it can constitute approximately 30% of total systemic circulation (compared to approximately 1% in healthy subjects) [246, 247]. Given that chronic microvascular occlusion is a feature of SCD, it is possible that the bronchial circulation undergoes expansion in response to loss of pulmonary vasculature. Given that patients with SCD must accommodate an elevated cardiac output, this expansion might be all the more pronounced and sufficient to cause airway oedema/compression.

3. Bronchoconstriction

Unmyelinated C-fibres are present throughout the lung parenchyma, bronchi and pulmonary vasculature. These fibres have been proposed as stretch receptors which respond to increased fluid load in the interstitium or the pulmonary/bronchial circulation [248, 249]. In canine models, distension of the pulmonary vasculature and experimental interstitial oedema were independently shown to cause increased C-fibre activity resulting in vagally-mediated reflex bronchospasm [248-251]. It is possible that similar process is taking place as a consequence of pulmonary vascular distension in SCD. In the children studied here, however, only a minority demonstrated bronchodilator responsiveness: of the 67 children with SCD studied in chapter five only four (6%) had a significant improvement in FEV₁ post bronchodilator. Furthermore, the magnitude of change in respiratory system resistance following bronchodilator did not differ between
SCD patients and controls. Of the children studied in chapter eight four SCD children (8.5%) in cohort one and seven (15.6%) in cohort two had a significant bronchodilator response at baseline. In the adult patients with SCD studied in chapter four, six (17%) had a significant response to bronchodilator. It is possible that C-fibre mediated reflex bronchoconstriction arising from distention of the pulmonary vessels was a factor in causing bronchospasm in those with significant reversibility, but the numbers of responders were too small to permit comparison of markers of pulmonary vascular volume between those patients who did and did not respond to bronchodilator.
9.2 **Longitudinal changes in lung function**

Lung function declines in children with SCD compared to similar aged, ethnic-matched controls. The most rapid period of deterioration appears to take place during early childhood. In the children with SCD studied in chapter eight, the occurrence of ACS was the sole independent predictor of a more rapid progression of pulmonary restriction. The key role of ACS is further supported by the fact that ACS episodes occurred more frequently per unit follow up period in the younger cohort one compared to cohort two; the annual rate of decline in lung volumes was also greater in cohort one. Airflow obstruction at baseline, but not asthma *per se*, was associated with a greater risk of developing ACS during the follow-up period. Obstructive abnormalities were much more common than restriction at baseline in both cohorts, but the prevalence of restrictive defects increased significantly over time. In cohort two, who were assessed to determine the long term changes in lung function as children with SCD transition to adulthood, the number of patients with restrictive defects at follow-up compared to baseline increased by 33% whilst the number with evidence of airflow obstruction remained relatively stable, decreasing by 6.2%.

In the subgroup of adults who were assessed longitudinally in chapter four, a different pattern of change was seen. For the group as a whole, TLC did not decline significantly over the follow-up period whereas significant reductions in FEV$_1$, VC, FEV$_1$/VC and FEF$_{25-75}$ were observed together with a significant increase in RV:TLC suggesting worsening airflow obstruction. Furthermore, an increase in small-vessel pulmonary vascular distension (as evidenced by the CSA<5mm%) was associated
with a more rapid decline in lung function. It should be noted, however, that the rate of decline in lung function in the adult cohort was considerably slower than that seen in the children. Compared to the adult cohort, the rate of decline in VC for the children of cohort one (chapter eight) was on average 3.2%/year faster. These data suggest an initial, rapid decline in lung volume in childhood with the rate of decline slowing as children age and that during adult life pulmonary restriction is relatively stable, with further gradual deterioration in ventilatory function mediated by continuing pulmonary vascular changes.
9.3 **Clinical implications**

The work presented in this thesis has implications for the clinical care of patients with SCD. Patients with SCD with recurrent wheezing or asthma have increased morbidity and mortality and it is therefore important to understand the underlying cause of the airflow obstruction that this represents in order that the correct treatment is used. Systemic and inhaled corticosteroids are an important part of the management of asthma, but are not likely to be efficacious in cases where airways disease is mediated by the pulmonary vasculature. The data presented in chapters four, five, six and seven have demonstrated that the airflow obstruction seen in SCD is, at least in part, due to changes in pulmonary vascular volume resulting from chronic anaemia and a hyper-dynamic circulation. In these circumstances, treatments directed at SCD itself such as hydroxyurea or transfusions are more likely to reduce respiratory morbidity. Indeed, systemic corticosteroids have been linked to an increase in painful crises and cerebrovascular accidents in patients with SCD [252, 253]. The need for correct treatment of airflow obstruction is emphasised by the results of the study described in chapter eight. Obstructive lung function abnormalities, regardless of a diagnosis of asthma, were predictive of subsequent ACS, and the occurrence of ACS was strongly associated with a more rapid decline in lung function and an increased likelihood of developing restrictive lung disease. The more rapid decline observed in younger children highlights the importance of the timing of therapy in preventing chronic lung disease in patients with SCD.
9.4 Future work

Further studies to determine the precise mechanisms underlying the link between vascular abnormalities and lung function would be beneficial to direct the development of possible future treatments. In chapter four, echocardiographic evidence of elevated left atrial filling pressure was presented and a significant correlation was observed with engorgement of the small pulmonary vessels (including veins). Since this may result in bronchial microvascular leakage and airway oedema, exploring the relationship between the pulmonary and bronchial circulation might provide further insight into the pathophysiology of lung function abnormalities in SCD (see section 9.1.1). The recent development and validation by Wanner et al of a non-invasive technique based on uptake of the soluble gas dimethyl ether to measure total bronchial blood flow [254] might provide a means to do this, especially if combined with echocardiography and imaging studies. Murine models expressing HbSS and employing micro-CT scanning might allow the impact of haemodynamic changes and fluid loading on the peripheral airways to be directly imaged, and since respiratory system resistance by forced oscillation can now be measured simply in mice the effect on lung function could also be explored. Assessment of the peripheral vasculature might also be helpful to determine if the pulmonary vascular changes described in this thesis are part of a generalised vasculopathy.

Large-scale longitudinal cohort studies would allow the results of physiological studies to be placed in a broader haematological context by assessing the relationships between lung disease and other complications such as renal and
hepatic dysfunction, and would allow the impact of lung function and pulmonary vascular abnormalities on important outcomes such as mortality, quality of life, and healthcare utilisation to be robustly assessed.

Data on the effect of common treatments on lung function abnormalities in SCD are also lacking. The data presented in this thesis suggest that treatments to reduce anaemia and increased pulmonary vascular volume or to prevent ACS, such as hydroxyurea or blood transfusion may be beneficial. The efficacy of these treatments will need to be assessed in future clinical trials.

9.5 Some general limitations regarding the presentation of lung function data

The results of lung function tests presented in this thesis have been expressed as the percentage of the predicted value for age, sex and height (where appropriate). Whilst this was commonplace during the period when this programme of study was designed [136], more recently the use of z-scores has been increasingly favoured [255], particularly in studies of paediatric populations. The presentation of z-scores carries some advantages over percentage predicted: chief amongst these is the fact that the z-score incorporates the effect of variation across age, sex and ethnicity [186, 190]. This facilitates the bias-free interpretation of within-individual serial measurements over the life-course and can be particularly helpful when examining graphical presentations of longitudinal lung function data. When the results of Chapter Four were being prepared for publication, the use of z-scores was still very
uncommon in studies of adult subjects and to facilitate comparison with existing
data, and on the advice of Prof Athol Wells (Royal Brompton and Harefield NHS
Foundation Trust) who has extensive experience reporting studies of pulmonary
structure-function relationships, we elected to present the lung function data as
percentage predicted. Subsequently, it was felt that it would aid clarity to maintain
a consistent presentation scheme across the thesis. Additionally, the studies
described in Chapters Five to Seven were also published using percentage
predicted data [208, 256-259], so it was decided to maintain consistency so far as
possible between thesis and published manuscripts. If the studies comprising this
thesis were to be conducted now, however, the wider cultural acceptance of the z-
score mode of presentation would make their use advisable and advantageous.
REFERENCES


10. Appendix A: publications arising from this thesis

10.1. Pulmonary Function, CT and echocardiographic abnormalities in sickle cell disease
Pulmonary function, CT and echocardiographic abnormalities in sickle cell disease

Alan Lunt,1,2 Sujal R Desai,3 Athol U Wells,4 David M Hansell,4 Sitali Mudumbai,3 Narbeh Melikian,3 Ajay M Shah,7,5 Swee Lay Tan,6,4,6 Anne Greenough1,2

ABSTRACT

Objectives: To test the hypothesis that vascular abnormalities on high-resolution CT (HRCT) would be associated with echocardiographic changes and lung function abnormalities in patients with sickle cell disease (SCD) and the decline in lung function seen in SCD patients.

Methods: HRCT, echocardiography and lung function assessments were made in 35 adults, 20 of whom had previously been assessed at a median of 6.6 years prior to this study. The pulmonary arterial dimensions on HRCT were quantified as the mean segmental pulmonary artery/brachial artery (A/B) ratio and the summed cross-sectional area of all pulmonary vessels ≤5 mm in diameter (cross-sectional area (CSA)≤5 mm²).

Results: The segmental A/B ratio was negatively correlated with FEV₁, vital capacity (VC), forced expiratory flow between 25% and 75% of VC (FEF₂₅₋₇₅%), and arterial oxygen saturation (SaO₂) and positively with the residual volume-total lung capacity ratio (RV/TLc) and respiratory system resistance (Rs). CSA≤5 mm² was negatively correlated with FEV₁, FEF₂₅₋₇₅%, and SaO₂ and positively with RV/TLc and respiratory system resistance (Rs). There were significant correlations between cardiac output assessed by echocardiography and the segmental A/B ratio and CSA≤5 mm². Lung function (FEV₁, p=0.0004; VC, p=0.0347; FEF₂₅₋₇₅%, p=0.0033) and the segmental A/B ratio (p=0.0347) and CSA≤5 mm² (p<0.0001) significantly deteriorated over the follow-up period.

Conclusions: Abnormalities in pulmonary vascular volumes may explain some of the lung function abnormalities and the decline in lung function seen in adults with SCD.

INTRODUCTION

Sickle cell disease (SCD) is one of the commonest inherited disorders worldwide, affecting an estimated 300,000 newborns every year.1 With improved general healthcare, the majority of patients with SCD in developed countries can expect to survive to adulthood. In adulthood, however, SCD can be associated with multiorgan damage, including pulmonary complications. Acute chest syndrome is the commonest cause of death in young adults, and pulmonary dysfunction is a major contributor to morbidity in aging adults with SCD. Lung function abnormalities are common in adults with SCD.2 Addons with SCD can suffer from parenchymal lung disease and pulmonary vascular disease or both, affected individuals can suffer premature death. Echocardiographic abnormalities consistent with raised pulmonary artery systemic pressure (PAP), suggestive of pulmonary hypertension in patients with SCD may be associated with increased morbidity and mortality.6-8 Right heart catheterisation studies, however, have demonstrated that only a proportion of SCD patients have pulmonary arterial hypertension (PAH), that is, elevated pulmonary arterial vascular resistance, and it is the presence of PH that is associated with early death.9,10 Nevertheless, elevated tricuspid valve regurgitant velocities demonstrated by echocardiography and thought to be suggestive of raised PAP are independently predictive of mortality.9,10

We have previously demonstrated that the majority of a cohort of adult patients with SCD had pulmonary abnormalities on high-resolution CT (HRCT).11 The HRCT findings significantly correlated with pulmonary function testing results; in particular, there were correlations between reductions in FVC and FEV₁, and the prominence of the central vessels on HRCT. Prominent central vessels were found on HRCT in eight of the nine patients.
Respiratory physiology

Figure 1. Measurement of cross-sectional area (CSA) - 5 mm² (A) using image software. (A) High-resolution CT image of lung field segmented with threshold values of -200 to -1024 HU. (B) Segmented image converted to binary image with window level of -750 HU, with pulmonary vessels displayed in black. (C) Mask image for particle analysis with vessel size set to 0-5 mm² and the circularity from 0.9 to 1.0.

METHODOLOGY

Patients and Methods

Adults with SCD were assessed between 2009 and 2013. Subjects included in the previous study had initially been assessed between 2003 and 2005. All participants gave written informed consent.

Lung function assessments

FEV₂, vital capacity (VC), forced expiratory flow between 25 and 75% of VC (FEF₂₅₋₇₅%), total lung capacity (TLC), residual volume (RV) and mean respiratory system resistance (ESR) were measured.

CT

CT patterns were quantified independently by two thoracic radiologists blinded to the clinical and functional data. The extent and severity of CT abnormalities were recorded in individual lobes, the lingula being considered a separate lobe for scoring purposes. Discal vessel dimensions, including both arteries and veins at the sub-segmental level, were measured using the method of Marshek et al. The total summed cross-sectional area for the vessels was then expressed as a percentage of the total lung area of the three selected slices using threshold values between -500 and -1024 HU (cross-sectional area (CSA) < 5 mm²/6) (figure 1). The mean segmental artery/binary (A/B) ratio in at least three of four lobes was calculated. CT total lung volume (TLCᵥ CT) was derived using a proprietary lung segmentation algorithm (figure 2). For the longitudinal analyses, initial and follow-up CT scans were compared.

Echocardiography

Two-dimensional and three-dimensional transthoracic echocardiography was performed according to the international guidelines. Examinations were performed using digital acquisition on a Philips-Sonos 7500 ultrasound system. Valvular regurgitation was graded from Doppler determinations of transvalvar flow. Tricuspid regurgitation (TR) was assessed in the parasternal right ventricular inflow, parasternal short axis and apical four-chamber views. A minimum of five sequential complexes were recorded. Continuous-wave Doppler signals of the peak regurgitant jet velocities (TRV) (normal < 2.5 m/s) were used to estimate the right ventricular systolic pressures (RVSP) using the Bernoulli equation 4 × R.V. + V² (normal < 80) and right ventricular diastolic volumes (RVDV) (normal range 100-160 mL) were also measured. Right and left ventricular function were assessed by measurement of the tricuspid annular plane systolic excursion (TAPSE) (normal > 1.3 cm) and the ratio of early diastolic flow (E) to lateral mitral annulus velocity (e') measured by tissue Doppler (E/e') (normal < 9). Technically acceptable TAPSE measurements were available in 32 patients and E/e' in 33 patients.

Statistical analysis

See online supplement.

RESULTS

Subjects

Thirty-five patients with a median age of 43 (range 17-73) years were assessed. In total, 29 of the 35 patients (median age at
Table 1 Lung function test results

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Mean (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;</td>
<td>77.4 (71.8-83.0)</td>
</tr>
<tr>
<td>VC</td>
<td>117.3 (111.0-124.0)</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;/VC</td>
<td>79.3 (75.9-83.0)</td>
</tr>
<tr>
<td>TC&lt;sub&gt;exp&lt;/sub&gt;</td>
<td>110.6 (105.0-118.0)</td>
</tr>
<tr>
<td>TLC</td>
<td>117.3 (111.0-124.0)</td>
</tr>
<tr>
<td>RV</td>
<td>117.3 (111.0-124.0)</td>
</tr>
<tr>
<td>RS/TLC</td>
<td>117.3 (111.0-124.0)</td>
</tr>
<tr>
<td>KCO</td>
<td>137.3 (125.0-148.0)</td>
</tr>
<tr>
<td>Res(0)</td>
<td>137.3 (125.0-148.0)</td>
</tr>
<tr>
<td>Res(&lt;1-s)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.059 (0.040-0.062)</td>
</tr>
<tr>
<td>SpO&lt;sub&gt;2&lt;/sub&gt; (%)</td>
<td>96.0 (95.0-97.0)</td>
</tr>
</tbody>
</table>

*Data are presented as the mean ± standard deviation. All measurements were made by trained technicians. The values are expressed as the mean ± standard deviation.*

Relationships between pulmonary function and HRCT results

Subpleural and subpleural lung consolidation were positively correlated with FEV<sub>1</sub> (r = 0.53, p = 0.0014), FEF<sub>25-75</sub> (r = 0.54, p = 0.0003), and the extent of a reticular pattern was negatively correlated with Res(0) (r = -0.51, p = 0.0319). TLC was positively correlated with FEV<sub>1</sub> (r = 0.51, p = 0.0014), VC (r = 0.48, p = 0.036), FEF<sub>25-75</sub> (r = 0.51, p = 0.036), and SpO<sub>2</sub> (r = -0.47, p = 0.037), and negatively with RV/TLC (r = -0.45, p = 0.007), Res(0) (r = 0.51, p = 0.0014), and Res(1) (r = 0.51, p = 0.0014).

The segmental A/B ratio and C<sub>5</sub> were negatively correlated with FEV<sub>1</sub> (r = -0.71, p = 0.0001), VC (r = -0.72, p = 0.0001), FEF<sub>25-75</sub> (r = -0.51, p = 0.0319), and SpO<sub>2</sub> (r = -0.44, p = 0.007), and positively with RV/TLC (r = 0.51, p = 0.0020), Res(0) (r = 0.69, p = 0.0001), Res(0) (r = 0.35, p = 0.00017), and Res(1) (r = 0.67, p = 0.0003).

The segmental A/B ratio and C<sub>5</sub> were independently related to a reduced FVC, VC, FEF<sub>25-75</sub>, and SpO<sub>2</sub> and to an increased RV/TLC, Res(0), and Res(1) (table 2). Linear bands were independently linked to a reduced FVC and TLC (table 2). The C<sub>5</sub> was independently linked to a reduced FEV<sub>1</sub>, VC, FEF<sub>25-75</sub>, SpO<sub>2</sub>, and to an increased RV, RV/TLC, Res(0), and Res(1) (table 3). The extent of a reticular pattern was associated with a reduced Res(0) and Res(1) (table 3).

Table 2 Results of multivariate analysis with the segmental A/B ratio as a predictor

<table>
<thead>
<tr>
<th>Lung Function</th>
<th>HRCT</th>
<th>β</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Segmental A/B ratio</td>
<td>-0.51</td>
<td>-0.57 to -0.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>VC</td>
<td>Segmental A/B ratio</td>
<td>-0.51</td>
<td>-0.57 to -0.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TLC</td>
<td>Segmental A/B ratio</td>
<td>-0.51</td>
<td>-0.57 to -0.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RV</td>
<td>Segmental A/B ratio</td>
<td>-0.51</td>
<td>-0.57 to -0.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RS/TLC</td>
<td>Segmental A/B ratio</td>
<td>-0.51</td>
<td>-0.57 to -0.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Res(0)</td>
<td>Segmental A/B ratio</td>
<td>-0.51</td>
<td>-0.57 to -0.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Res(1)</td>
<td>Segmental A/B ratio</td>
<td>-0.51</td>
<td>-0.57 to -0.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SpO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Segmental A/B ratio</td>
<td>-0.51</td>
<td>-0.57 to -0.45</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*HRCT, high-resolution CT; C<sub>5</sub>, C<sub>5</sub>; ground glass opacification; RV/TLC, residual volumetric lung capacity ratio; VC, vital capacity.
Respiratory physiology

Table 3  Results of the multivariate analysis with CSA <5 mm² as a predictor

<table>
<thead>
<tr>
<th>Lung function</th>
<th>HRCT A (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FV</td>
<td>CSA &lt;5 mm² 0.52 -0.60 (-0.94 to -0.10) &lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>VC</td>
<td>0.62 (-0.49 to -0.39) 0.0003</td>
<td></td>
</tr>
<tr>
<td>TLC</td>
<td>0.51 -0.71 (-1.06 to -0.36) 0.0029</td>
<td></td>
</tr>
<tr>
<td>RV</td>
<td>0.26 -0.76 (-1.06 to -0.46) 0.0002</td>
<td></td>
</tr>
<tr>
<td>RV/TLC</td>
<td>0.11 -0.36 (-0.75 to -0.03) 0.0486</td>
<td></td>
</tr>
<tr>
<td>RV/VC</td>
<td>0.36 0.15 (-0.23 to 0.62) 0.0009</td>
<td></td>
</tr>
<tr>
<td>RV/SLC</td>
<td>0.40 0.20 (0.09 to 1.00) 0.0000</td>
<td></td>
</tr>
<tr>
<td>TLC/SLC</td>
<td>0.14 0.42 (0.12 to 0.72) 0.0058</td>
<td></td>
</tr>
<tr>
<td>RV/TLCO</td>
<td>0.50 -0.51 (-0.80 to -0.22) 0.0010</td>
<td></td>
</tr>
<tr>
<td>RV/TLCO</td>
<td>0.40 -0.59 (-0.83 to -0.18) 0.0001</td>
<td></td>
</tr>
</tbody>
</table>

*All correlations were significant at p<0.05.

Echocardiography results
The median TVR was 2.65 (range 1.59-4.69) (m/s²), the median estimated PAP 13.3 (range 9.0-20.0) (mmHg), the mean CO 6.0 (range 3.3-9.1) (L/min) and the median RVDV 93.9 (range 36.7-212.8) (mL/dL). The median TAPSE was 2.10 (1.00-2.75) (cm), and the median E′/e ratio 1.10 (0.40-1.40). Significant correlations were observed between CO and A/B (r=0.41, p=0.013) and the CSA-C <5 mm² (r=0.55, p=0.004), but not with the estimated PAP. There was a correlation of CSA-C <5 mm² with E′ (r=0.45, p=0.009). There was also a correlation of CSA-C <5 mm² with TAPSE (r=0.38, p=0.031), but only one subject had an abnormal TAPSE result (<1.5) and thus we cannot comment on the relationship between HRCT indices of vascular dilatation and echo indices of RV dysfunction.

The segmental A/B ratio and the CSA-C <5 mm² were significantly correlated with the haemoglobin levels (r=0.56, p=0.0011) and the lactate dehydrogenase (LDH) levels (r=0.50, p=0.0049). The CSA-C <5 mm² correlated with the reticulocyte count (r=0.39, p=0.0147).

Longitudinal analysis
The lung function results of the 20 patients who were reassessed had declined significantly (table 4). There were, however, no significant changes seen in the prevalence or extent/ severity of lung parenchymal abnormalities on CT, but both the median segmental A/B ratio and CSA-C <5 mm² had increased significantly over the follow-up period (table 3). The percentage change from baseline in the A/B ratio correlated negatively with that in FEV₁ (r=0.30, p=0.0136) and carbon monoxide transfer coefficient, corrected for haemoglobin concentration (KCO) (r=0.55, p=0.0310) and positively with that in RV (r=0.47, p=0.0027) and TLCO (r=0.57, p=0.0047) and negatively with RV/TLC (r=0.37, p=0.0379) and positively with RV/TLC (r=0.57, p=0.0047) and TLCO (r=0.57, p=0.0047). Logistic regression demonstrated that a higher CSA-C <5 mm² at baseline was predictive of a subsequent overall deterioration in HRCT appearance, that is, a gold change score of 1 (OR 2.62 (95% CI 1.14 to 6.01) per unit increase in CSA-C <5 mm², p=0.023). Linear mixed model analysis demonstrated a significant association of baseline CSA-C <5 mm² with subsequent decline in TLC, with an increased decline of −1.84% (95% CI −3.31 to −0.37) per year for each unit increase in CSA-C <5 mm² at baseline, p=0.017. CSA-C <5 mm² was also predictive of a more rapid change in RV/TLC, with a change in slope of +4.93% (95% CI 0.88 to 8.93) per year per unit increase in CSA-C <5 mm² at baseline, p=0.017.

DISCUSSION
We have demonstrated that pulmonary vascular abnormalities on HRCT were significantly related to pulmonary function impairment in adults with SCD. The segmental A/B ratio and CSA-C <5 mm² were independently linked to reductions in FEV₁, VC and TLCO, and to increased respiratory system resistance and RV/TLC. In addition, small vessel size correlated with reduced oxygen saturation and haemoglobin concentration and increased LDH, bilirubin and reticulocyte levels. These results suggest relationships between anaemia, haemolysis, hypoxia and pulmonary function abnormalities. We found a positive correlation between CSA-C <5 mm² (a measure of distal arterioles and veins) and E′/e which is a marker of left atrial filling pressure that is elevated if there is LV diastolic dysfunction. A higher E′/e would result in some elevation of pulmonary venous pressure, and this result suggests a role for pre-capillary and post-capillary pulmonary vascular changes in SCD-related lung

Table 4  Pulmonary function results at initial and follow-up assessment

<table>
<thead>
<tr>
<th>2005-2007</th>
<th>2008-2010</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV₁</td>
<td>86.6 (43.9-115.4) 20.9 (5.5-48.6) 0.0004</td>
<td></td>
</tr>
<tr>
<td>VC</td>
<td>85.3 (48.4-102.3) 74.5 (27.1-166.2) 0.0047</td>
<td></td>
</tr>
<tr>
<td>RV/TLC</td>
<td>15.4 (30.1-129.3) 59.7 (74.0-113.0) 0.0021</td>
<td></td>
</tr>
<tr>
<td>TLCO</td>
<td>77.1 (33.1-160.0) 73.3 (18.0-210.9) 0.0003</td>
<td></td>
</tr>
<tr>
<td>RV</td>
<td>87.6 (46.7-135.9) 70.0 (93.1-136.2) 0.0113</td>
<td></td>
</tr>
<tr>
<td>RV/TLC</td>
<td>15.4 (30.1-160.0) 11.1 (5.6-195.0) 0.0053</td>
<td></td>
</tr>
<tr>
<td>TLCO</td>
<td>77.1 (33.1-160.0) 73.3 (18.0-210.9) 0.0003</td>
<td></td>
</tr>
<tr>
<td>RV/TLC</td>
<td>15.4 (30.1-160.0) 11.1 (5.6-195.0) 0.0053</td>
<td></td>
</tr>
</tbody>
</table>

*Data are expressed as median (range) and presented as per cent predicted for healthy age and sex unless indicated. RV/TLC, residual ventilated lung capacity ratio; VC, vital capacity.

Table 5  High-resolution CT (HRCT) parenchymal and vascular analysis at initial and follow-up assessment

<table>
<thead>
<tr>
<th>HRCT pattern</th>
<th>Initial</th>
<th>Follow-up</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pattern</td>
<td>Extent/severity</td>
<td>Extent/severity</td>
<td></td>
</tr>
<tr>
<td>Bilateral pattern</td>
<td>5.0 (6.0-17.8)</td>
<td>5.0 (6.0-17.8)</td>
<td>0.1987</td>
</tr>
<tr>
<td>Ground glass opacification</td>
<td>0 (0-3)</td>
<td>0 (0-3)</td>
<td>0.3528</td>
</tr>
<tr>
<td>Decreased intensity</td>
<td>1.0 (0-14.7)</td>
<td>1.0 (0-14.7)</td>
<td>0.9973</td>
</tr>
<tr>
<td>Consolidation</td>
<td>0.0 (0-4.2)</td>
<td>0.0 (0-4.7)</td>
<td>0.5000</td>
</tr>
<tr>
<td>Lobular volume loss</td>
<td>1.0 (0-7.7)</td>
<td>1.0 (0-7.7)</td>
<td>1.0000</td>
</tr>
<tr>
<td>Linear bands</td>
<td>1.0 (0-5.0)</td>
<td>1.0 (0-5.0)</td>
<td>0.9918</td>
</tr>
<tr>
<td>Subpleural connective lines</td>
<td>0.0 (0-2)</td>
<td>0.0 (0-2)</td>
<td>0.9080</td>
</tr>
<tr>
<td>CSA-C &lt;5 mm²</td>
<td>0.25 (0.14-55)</td>
<td>0.54 (0.22-11.0)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

*Data are presented as median (range).
There was a negative relationship with vascular dimensions and VC, FEV₁, FEF₂⁰, and SpO₂ and a positive relationship with RV, RV/TLC, Res(0) and Res(1), suggesting vascular dimensions were related to an obstructive defect. VC, FEV₁ and FEF₂⁰, however, can be reduced in both obstruction and restriction and thus the correlations with markers of vascular dilation and reductions in VC, FEV₁ and FEF₂⁰ may also indicate a relationship between vascular dilation and the development of restrictive lung function.

In our cohort, 23% of patients had evidence of a restrictive lung function defect and 17% had evidence of an obstructive defect. These results are consistent with those of Sarntti et al but differ from those of Klinge et al, who found a much lower incidence of airflow obstruction. The differences may be due to a number of factors. We used a recently reported ethnic-specific reference range for spirometry, whereas Klinge et al used a Caucasian reference range with fixed correction factors to adjust for ethnicity. We classified abnormalities based on the lower limit of normal based on percentiles recommended by the American and European Thoracic Societies, whereas Klinge et al used a fixed percentage predicted value to define the lower limit of normal for all lung function indices, which does not take into account that the limits of the normal range vary with age, sex and ethnicity. Furthermore, the classification scheme in the Klinge’s study specified that in order to be classified as obstructive or mixed, the DLCO had to be normal, which precluded the possibility that impaired gas transfer coexisted with airflow abnormalities.

Restrictive lung function defects, that is, a reduced TLC and RV were not associated with the extent of ground glass opacification on a reticular pattern and showed only a modest association with linear bands, suggesting interstitial fibrosis may not be the predominant mechanism for loss of lung volume in patients with SCD. Indeed, most CT markers of pulmonary fibrosis showed no association with reductions in VC, FEV₁, FEF₂⁰, RV/TLC, SpO₂, Res(0) or Res(1). The extent of a reticular pattern was associated with a reduction in respiratory system resistance; we speculate that this might be due to the tractional effects of areas of fibrosis on adjacent bronchi. Subpleural curvilinear bands were noted in 24% of patients; this is an unusual CT sign, formerly believed to be pathognomonic of early asbestosis. Subpleural curvilinear bands have subsequently been described in association with micro-infections in patients with interstitial defect and respiratory muscle weakness and as reversible sign caused by interstitial oedema resulting from pulmonary congestion. Observations in subjects without lung disease and undergoing lymphodrainage have led to the suggestion that the sign may represent an engorged subpleural lymphatic network. Given the presence of a high-output state in SCD, and the correlation of subpleural curvilinear lines with markers of small-vessel dilatation observed in our cohort, it is tempting to speculate that this pattern may be related to interstitial oedema and/or increased lymphatic drainage.

We highlight a decline in lung function over a mean of 6.7 years in adults with SCD. A significant decrease was observed in VC, FEV₁, FEF₂⁰, FEV₁/VC and KCO with a significant increase in RV/TLC. There were no significant changes in the results of the CT assessments other than in vascular dimensions, wherein both the A/B ratio and CSA×S mm²/mm significantly increased. These results suggest changes in pulmonary vascular dimensions may be responsible for the decline in lung function. Furthermore, a greater baseline CSA×S mm²/mm was predictive of a more rapid progression of both obstructive (increasing RV/TLC) and restrictive (decreasing TLC) lung disease and an increased likelihood of a deterioration in pulmonary disease, as evidenced on HRCT examination. Our results emphasise the phenotypic heterogeneity of SCD lung disease. The changes in vascular morphology related to obstructive defects and the likelihood of deteriorating interstitial lung disease, suggesting that there may be a shared mechanism involving small pulmonary vessels. Fieldt et al demonstrated that bone marrow-derived fibroblasts may be mobilised into the circulation and subsequently extravasate into the lungs of SCD mice. There they function as mesenchymal progenitor cells for the production of extracellular matrix and contribute to the development of fibrosis. Elevated levels of circulating fibrocytes have been observed in humans with SCD. We, therefore, speculate that pulmonary vascular engorgement and distension may potentiate extravasation of circulating fibrocytes.

This study has strengths, but some limitations too. A strength of this study was the use of two different quantitative methods for assessing pulmonary vascular morphology, the segmental A/B ratio and CSA×S mm²/mm, which yielded similar results. We used ethnic-specific references for spirometric indices, but the static lung volume results were related to reference ranges derived from Caucasian subjects with a fixed correction factor to account for ethnicity. All the study population were African or Caribbean, thus correlations within the cohort and comparisons between results at baseline and follow-up were valid. A limitation is that we did not have right heart catheterisation data for pulmonary artery pressures and resistance, but all the patients underwent the same echocardiographic protocol. Studies have demonstrated a minority of patients with elevated TRV have elevated pulmonary artery pressure. In a recent study, only 8 of 26 patients with elevated TRV at echocardiography had elevated pulmonary artery pressure confirmed by right heart catheterisation and similar findings were reported in a larger study with 10.4% of 243 patients with elevated TRV having pulmonary hypertension. It should be noted, however, that a TRV of >2.5 m/s (corresponding to an estimated mPWP greater than or equal to approximately 2 SDs above normal) does not meet the criteria for right-heart catheter-defined pulmonary arterial hypertension (mPWP >23 mm Hg), which is 3 SDs above normal. The latter corresponds to a TRV of approximately 3.6 m/s, which occurs in only about one-third of patients. Different HRCT protocols were used in our earlier study, and in this study, however, care was taken to ensure that all the images used for comparisons were from anatomically comparable sections. In addition, scoring was undertaken by observers who were blinded to the results of lung function and echocardiography results. The consistent relationship between CSA×S mm²/mm, A/B ratio and lung function test results suggests the different protocols did not adversely influence our results. We have shown that, if TCLCT is measured, volumetric HRCT scans are able to capture both restrictive and obstructive functional abnormalities, providing an alternative method to assess global pulmonary impairment in patients with SCD.

In conclusion, we have demonstrated an association between small-vessel pulmonary vascular dimensions on HRCT reflecting pulmonary vascular volume, lung function abnormalities and echocardiographic estimates of CO and ventricular function in adults with SCD. Our results suggest that abnormalities in pulmonary vascular volume may explain some of the lung function abnormalities and the decline in lung function seen in adults with SCD.

Contributors AG, SB, AW, DJM, AMG and ST were involved in the design of the study. AG, SB, DVM and ST were involved in the acquisition of data. All authors were involved in the analysis of the data and the preparation of the manuscript.
Respiratory physiology

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Disclaimer: The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

Ethics approval: The study was approved by King’s College Hospital Ethics Committee (REC code 02/08/003).

Provenance and peer review: Not commissioned; externally peer reviewed.

Data sharing statement: We agree with the data sharing statement.

REFERENCES

10.2. Lung function, transfusion, pulmonary capillary blood volume and sickle cell disease

Lung function, transfusion, pulmonary capillary blood volume and sickle cell disease

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b National Institute for Health Research (NIHR) Respiratory Biomedical Research Centre based at Guy’s and St. Thomas’ NIHR Foundation Trust and King’s College London, UK
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A B S T R A C T

Lung function abnormalities occur in children with sickle cell disease (SCD) and may be associated with elevated pulmonary blood volume. To investigate that association, we determined whether blood transfusion in SCD children acutely increased pulmonary capillary blood volume (PCBV) and increased respiratory system resistance (RV/VC). Measurements of RV and RV/VC were made before and after blood transfusion in 18 children, median age 14.2 (6.6–18.5) years. Diffusing capacity for carbon monoxide and arterial oxygen were measured before and after transfusion. The median RV increased from 123.4 to 141.3% predicted (p = 0.0001) and pulmonary capillary blood volume from 38.7 to 64.1 ml/m² (p = 0.0001). A forced expiratory volume in one second (FEV1) was measured before and after transfusion. RV/VC increased from 54.0 to 60.3% predicted (p = 0.02). The increase in RV correlated with the increase in PCBV (r = 0.56, p = 0.003). Increased pulmonary capillary blood volume may at least partially explain the lung function abnormalities in SCD children.

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1. Introduction

Sickle cell disease (SCD) is one of the commonest inherited disorders worldwide; approximately 250,000 children are born with homozygous SCD per year (Hed et al., 2011). The majority of children with SCD in developed countries can expect to survive to adulthood (Quinn et al., 2010), but may then suffer severe pulmonary morbidity including hypoxia and pulmonary hypertension (Powars et al., 1989). Restrictive and obstructive lung function abnormalities are common. Restrictive lung disease is associated with increasing age (Sylvester et al., 2004; Maclean et al., 2003) and obstructive defects can occur even in young children with SCD (Kouninbours et al., 2001, 1997). Hence, it is important to understand the etiology of such abnormalities: mechanistic insights are necessary to better inform therapy to hopefully prevent SCD pulmonary complications. A number of researchers have suggested that asthma is responsible for impaired lung function and respiratory symptoms in SCD, but the evidence is conflicting. A high prevalence of asthma in children with SCD has been reported in one study (Knight-Madden et al., 2005), but not in others (Bernaudin et al., 2009; Boyd et al., 2004). In addition, the response to bronchodilators challenges in SCD patients, such as cold air, exercise, or methacholine has been variable ranging from no response (Chaudry et al., 2010) to 78% of bronchodilator having a positive response (Cleick et al., 2007). Furthermore, whereas some studies have shown a high response to bronchodilator therapy in SCD children compared to controls (Knight-Madden et al., 2005; Kouninbours et al., 2003), others have shown no significant difference in the response rates of SCD children compared to that of controls (Sylvester et al., 2004).

An alternative explanation for the impaired lung function is increased pulmonary capillary blood volume due to chronic anemia resulting in a raised cardiac output and increased vascular recruitment and distension (Bates et al., 2002; Chaudry et al., 2011; Deluca et al., 2005; Henri-Pierre et al., 1970; Lunt et al., 2014; Wedderburn et al., 2014). Indeed, we have demonstrated children with SCD have increased pulmonary capillary blood volume compared to controls, which was associated with airway obstruction and correlated with respiratory system resistance.

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Table 1

<table>
<thead>
<tr>
<th>Pro-trans fusion</th>
<th>Post-trans fusion</th>
<th>% Change from baseline</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual blood</td>
<td>173.4 (88.6-276.4)</td>
<td>163.8 (86.5-239.8)</td>
<td>-4.5 (1.6-7.3)</td>
</tr>
<tr>
<td>Residual blood</td>
<td>22.4 (8.3-37.5)</td>
<td>24.8 (10.5-62.8)</td>
<td>-7.5 (3.5-11.7)</td>
</tr>
<tr>
<td>Residual blood</td>
<td>3.5 (0.3-6.5)</td>
<td>4.0 (0.3-6.5)</td>
<td>-2.5 (0.5-4.5)</td>
</tr>
<tr>
<td>Residual blood</td>
<td>1.27 (0.47-3.18)</td>
<td>1.37 (0.67-2.48)</td>
<td>-0.7 (0.2-1.2)</td>
</tr>
<tr>
<td>Residual blood</td>
<td>0.09 (0.04-0.33)</td>
<td>0.08 (0.02-0.26)</td>
<td>-0.3 (0.0-0.7)</td>
</tr>
<tr>
<td>Residual blood</td>
<td>24.6 (7.3-139.6)</td>
<td>50.4 (20.1-136.7)</td>
<td>-2.6 (1.0-4.2)</td>
</tr>
<tr>
<td>Residual blood</td>
<td>94.1 (7.1-145.1)</td>
<td>100.7 (85.8-139.8)</td>
<td>-6.6 (0.5-12.7)</td>
</tr>
<tr>
<td>Residual blood</td>
<td>4.0 (1.6-6.5)</td>
<td>4.0 (1.6-6.5)</td>
<td>-0.5 (0.0-1.0)</td>
</tr>
<tr>
<td>Residual blood</td>
<td>103.2 (90.3-116.2)</td>
<td>106.4 (90.3-116.2)</td>
<td>-3.8 (0.0-7.6)</td>
</tr>
<tr>
<td>Residual blood</td>
<td>74.9 (52.2-96.1)</td>
<td>74.9 (52.2-96.1)</td>
<td>-0.1 (0.0-0.2)</td>
</tr>
<tr>
<td>Residual blood</td>
<td>7.4 (5.1-9.1)</td>
<td>7.4 (5.1-9.1)</td>
<td>-0.1 (0.0-0.2)</td>
</tr>
<tr>
<td>Residual blood</td>
<td>92.7 (71.6-115.6)</td>
<td>92.7 (71.6-115.6)</td>
<td>-0.1 (0.0-0.2)</td>
</tr>
<tr>
<td>Residual blood</td>
<td>90.1 (41.7-194.1)</td>
<td>90.1 (41.7-194.1)</td>
<td>-0.1 (0.0-0.2)</td>
</tr>
<tr>
<td>Residual blood</td>
<td>55.5 (39.0-73.6)</td>
<td>55.5 (39.0-73.6)</td>
<td>-0.1 (0.0-0.2)</td>
</tr>
<tr>
<td>Residual blood</td>
<td>66.3 (57.1-80.3)</td>
<td>66.3 (57.1-80.3)</td>
<td>-0.1 (0.0-0.2)</td>
</tr>
<tr>
<td>Residual blood</td>
<td>64.1 (53.4-72.6)</td>
<td>64.1 (53.4-72.6)</td>
<td>0.1 (0.0-0.2)</td>
</tr>
</tbody>
</table>

* The results are expressed as median (range) and percent predicted for height except where indicated.

* Indicates absolute change.

(Wedelthorpe et al., 2014). Similarly in adults, vascular changes on high-resolution computed tomography (HRCT) increased segmental pulmonary artery diameter and total cross-sectional area of all pulmonary vessels less than 5 mm in diameter (CSA < 5 mm²), correlated with reductions in lung function (Gust et al., 2013).

Experimental increases in thoracic blood volume have been shown to produce reductions in lung function and an increase in respiratory system resistance. For example, rapid saline infusion has been shown to produce a significant decrease in dynamic lung volumes as assessed by spirometry in healthy adults (Collins et al., 1975), as well as in patients with left ventricular failure (Juni et al., 1993). In addition, Loring et al. found that the inflation of pneumatic neumotests in healthy subjects caused an increase in respiratory system resistance as assessed by impulse oscillometry (Loring et al., 1993). Recently, Shah et al. demonstrated that in healthy subjects, inflation of 6% saline caused a significant increase in respiratory system resistance at 5% as assessed by impulse oscillometry. Such changes have not been observed in SCD children, but some SCD children receive blood transfusions as part of their routine care. Blood transfusions have been shown to result in transient increases in cardiac output (Duke et al., 1995). Routine blood transfusions in SCD children would therefore allow investigation of the acute effect of fluid loading on lung function and further add to the understanding of the epidemiology of pulmonary function impairment in SCD children. As a result, this study was to test the hypothesis that blood transfusion would result in an acute increase in pulmonary capillary blood volume and respiratory system resistance and reductions in spirometry in children with SCD. Such data would further add to the understanding of the pathophysiology of lung function abnormalities in SCD children.

2. Methods

Children homozygous for sickle cell haemoglobin (HbSS) undergoing regular blood transfusion at King’s College Hospital NHS Foundation Trust, London were recruited. Only children of seven years of age or greater were recruited as they were likely to be able to complete all the lung function tests. The study was approved by the King’s College Hospital NHS Foundation Trust Research Ethics Committee and parents gave informed, written consent for their child to take part. Impulse oscillometry, spirometry and pulmonary capillary blood volume were measured before and immediately after transfusion. In order to further characterise the lung function of the patients, static lung volumes were measured before transfusion. The volume of packed red cells (PRC) administered to each patient was determined using the following formula: PRC (ml) = patient weight (kg) × change in haemoglobin to be achieved (g/dl) × transfusion factor (4.5 ml/kg g/dl⁻¹), in order to achieve a target haemoglobin concentration of 13.5 g/dl. All transfusions were administered over approximately four hours.

2.1. Lung function assessments

Spirometry, gas transfer for carbon dioxide and body plethysmography were performed. The forced expiratory volume in one second (FEV1) vital capacity (VC), ratio of FEV1 to VC (FEV1/VC), forced expiratory flows (FEF25-75 and FEF75-125), transfer factor for carbon monoxide (TlCO), transfer factor adjusted for alveolar volume (Kco), total lung capacity (TLC), residual volume (RV) and the ratio of RV to TLC (RV/TLC) were assessed according ATS/ERS guidelines. The highest forced vital capacity result obtained from spirometry and the slow vital capacity from the bronchoalveolar lavage were reported as RV/TLC. Results were expressed as the percentage predicted for height using an ethnic specific reference range for spirometry and Caucasian reference data for gas transfer and body plethysmography (Baan et al., 2004). The percentage predicted for height and the predicted percentage predicted for height were calculated using the reference ranges for height and predicted height.

Residual volume (RV) was also measured using impedance plethysmography. RV was measured before the other lung function tests and a resistance at 5 Hz (Rs5) was used in order to assess both distal and proximal changes in lung function. Respiratory system resistance at 20 Hz (Rs20), the frequency dependence of resistance (Rs5–Rs20), the respiratory system resistance at 5 Hz (Res5), the resonant frequency (Fre) and the area under the resonance curve between resonant frequency and 5 Hz (A) were also recorded. For all 106 indices the whole-breath values were reported. All measurements were performed using a commercially available lung function system (Jaeger MasterScreen IOS, Carefusion Ltd, Eastleigh, UK).

The Rs5, Rs20, SpO2, and flow results were expressed as the percent predicted for height using the reference ranges of (Weinberger et al., 2005). Raw values were reported for Rs5–Rs20 and A as predicted values are not available for these indices for the age range of the children studied. For IOS indices, the mean of two measurements for Rs5 and Rs20 were reported for each measurement the two results were within 5% of each other.

Patients were diagnosed with an obstructive abnormality if their FEV1/FVC was less than the lower limit of normal (LLN)
Fig. 1. RBC and pulmonary capillary blood volume before and after transfusion. Individual's data are shown by linked data points. Patients with spirometrically-defined obstructive defects at baseline are shown by dashed lines.
2.2. Pulmonary capillary blood volume

Pulmonary capillary blood volume was measured using the single-breath-hold method for gas transfer for carbon monoxide (DLCO) and nitric oxide (DLNO) in a commercially available system (Jaeger MasterScreen PFT Pro Carefusion Ltd. Basingstoke UK). Pulmonary membrane diffusing capacity (DMCO) and pulmonary capillary blood volume (PCBV) were determined using the Roughton-Furter model as previously described (Wroblewski et al., 2014). PCBV was "normalised" to estimated body surface area (BSA) using the formula of Montefeltre, 1987. The mean of two measurements within 5% was reported. Baseline DLCO results corrected for the haemoglobin concentration (Hb) from a blood test taken immediately before transfusion were also recorded.

3. Sample size

18 children studied pre- and post-transfusion allowed detection of differences before and after transfusion equivalent to one standard deviation of the results of each measurement technique with 80% power at the 5% level.

4. Analysis

The data were not normally distributed as assessed by the Pearson Chi-squared test. Therefore, differences were assessed using Mann-Whitney U-tests. Spearman’s correlations were calculated to determine the strength of relationships. Statistical analysis was performed using R software version 3.1.1 (Foundation for Statistical Computing, Vienna, Austria).

5. Results

Eighteen subjects with a median age of 14.2 (range 6.8-18.5) years were assessed. Their median haemoglobin was 10.1 (range 8.2-11.5) g/dl and the median packed red cell volume (PCV) administered was 500 (250-400) ml. Eight children (44%) had at least one acute chest syndrome episode and four (22%) had a physician’s diagnosis of asthma.

Prior to transfusion, their median TcPCV was 92.2 (71.6-135.1)% predicted for height and RV/TLc was 1051 (78.4-1434.3) predicted for height. Six children had an obstructive (33%) and two (11%) a restrictive defect. Pulmonary capillary blood volume (PCBV) (Fig. 1), RsNO (Fig. 1), RsNO, 85-I2O, Xe5, AX, EICO and RCO all increased after transfusion (p < 0.0001, p < 0.0001, p < 0.0332, p < 0.0082, p = 0.0023, p = 0.0052, p < 0.0001, p < 0.0001, respectively) (Table 1), whereas FIVC, VC and IT25-75 all declined (p = 0.0066, p = 0.0008, p = 0.0483 respectively) (Table 1). No significant change was seen in free (p = 0.0665) or FIV/CVC (p = 0.2963). The percentage change from baseline in pulmonary capillary blood volume correlated with the change in RsNO (r = 0.55, p < 0.0049), Rs2O (r = 0.60, p = 0.0091) and RCO (r = 0.58, p = 0.0265), but not with 85-I2O (r = 0.34, p = 0.161), EICO (r = 0.43, p = 0.0981), VC (r = 0.37, p = 0.1279), IT25-75 (r = 0.24, p = 0.3366), FIV/VC (r = 0.24, p = 0.3280) or EICO (r = 0.31, p = 0.1876). The change in pulmonary capillary blood volume was correlated with the PCV (r = 0.56, p = 0.0155).

6. Discussion

We have demonstrated significant changes in lung function immediately following blood transfusion in children with SCD, increases in respiratory system resistance, reactance and gas transfer decreases in FIV/CVC. Pulmonary capillary blood volume increased significantly and this change was correlated with changes in RsNO, Rs2O and RCO, VC also decreased, resulting in no significant change in FIV/CVC.

Compared to healthy controls, children with SCD have an elevated cardiac output and pulmonary blood flow at rest, as well as an increased pulmonary blood volume as a result of chronic anemia (Chaudhry et al., 2014; Wroblewski et al., 2014). Our results suggest a link between increased pulmonary vascular volume and lung function abnormalities in SCD and that it is possible that respiratory symptoms reported in some children with SCD may have been incompletely attributed to asthma. It is important to emphasize that these results are not highlighting blood transfusions are inappropriate for SCD children. Indeed, the blood transfusions were associated with an increase in DLCO and RCO indicating improved gas exchange. Investigating long term function and capillary blood volume before and after blood transfusion has a further means of investigating the association of pulmonary mechanics, lung function abnormalities and pulmonary capillary blood volume.

This study has strengths and some limitations. The main strength of this study was the use of routine transfusion as a means of fluid loading, a treatment likely to alter pulmonary blood volume. This provided an “experimental” model which allowed lung function to be assessed at different pulmonary capillary blood volumes, which would be inappropriate to do by other methods in SCD children. Although evidence of a possible relationship between FIV/CVC, VC and IT25-75 all decreased, however, the magnitude of change was small and not consistent with clinical importance. Therefore, the results should be interpreted with caution and further studies are needed to confirm these findings.

In conclusion, the results of this study indicate that blood transfusion in children with SCD may result in significant changes in lung function, particularly respiratory system resistance and reactance, and gas transfer. These changes may be related to the increased pulmonary capillary blood volume associated with blood transfusion. Further research is needed to investigate the long-term effects of blood transfusion on lung function and pulmonary mechanics in children with SCD.
both pre- and post-transfusion measurements, paired comparisons between them were valid.

7. Conclusions

In conclusion, this study has demonstrated that significant increases in pulmonary capillary blood volume and respiratory system resistance occur immediately following blood transfusion in children with SCD. Furthermore, the increase in respiratory system resistance significantly correlated with the increase in pulmonary capillary blood volume. These results provide evidence for a potential interaction between the increased pulmonary capillary blood volume and pulmonary function abnormalities seen in SCD children.

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Conflict of interest

None to declare.

Acknowledgement

We thank Mrs Deirdre Gibbons for secretarial support.

References

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

Alan Lunt, Naleem Ahmed, Gerard F. Rafferty, Miola Dick, David Rees, Sue Height, Swy Lay Thein and Anne Greenough

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, elevated nitric oxide (FENO) is a surrogate marker for obstructive lung disease. However, we have also been 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 < 0.0001), alveolar NO production (P < 0.0001), and pulmonary blood flow (P < 0.0001), but not airway NO flux. There was no significant correlation between FENO and respiratory system resistance in either group, but in the SCD children, there were correlations between alveolar NO production (P < 0.0001) and concentration (P < 0.0001) and pulmonary blood flow.

CONCLUSION: Airway NO flux was not elevated in the SCD children, nor correlated with airway obstruction, suggesting that airway 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. 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 airway obstruction in SCD is the hyperdynamic pulmonary circulation due to a raised cardiac output resulting from chronic anemia (4). 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 scans, we used two quantitative measures to assess vessel dilatation (the segmental artery-branching (A/B) ratio and the total cross-sectional area of all pulmonary vessels less than 5 mm in diameter (cross-sectional area < 5 mm²)) (10). Increases in segmental A/B ratio and cross-sectional area < 5 mm² were independently linked to reductions in forced expiratory volume in 1 s (FEV1), vital capacity (VC), and forced expiratory flow between 25 and 75% of VC (FEF25-75%) and to increased respiratory system resistance and residual volume-total lung capacity (RV/TLCO). 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 (FENO), 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, FENO 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 FENO, however, has not been found in other cohorts (13,14). FENO 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 FENO 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) influence their FENO levels has not been investigated.

In individuals with SCD, FENO has been previously measured at a single fixed exhalation flow of 30 ml/s (11,13) but measurements at multiple exhalation flows allow partitioning into...
flow-independent airway and alveolar components (18,19).
Using such a technique, SCD children were found to have an elevated
airway nitric oxide flux, but the alveolar NO concentration did not differ significantly from that found in healthy
controls (14). A limitation of this study (14), however, was that
the children in the control group were not matched for race and
ethnic differences have been reported for \( F_{NO} \) indices (20–22).
Furthermore, as the transfer factor for NO (DLmNO) was not
measured (14), it was not possible from those results to deter-
mine whether any elevation might be due to passive accumula-
tion due to gas exchange impairment or reflected increase in
alveolar production. Thus, it is important that flow-independent
\( F_{NO} \) parameters obtained using the multiple expiration flow
method are measured in SCD patients and compared with
appropriately matched controls to reliably determine whether
or not \( F_{NO} \) is elevated in SCD children and whether any eleva-
tion of \( F_{NO} \) is due to increased airway NO flux or to increased
alveolar NO production due to a hyperdynamic pulmonary cir-
culation.

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 cor-
related with airways obstruction and alveolar NO production to
a hyperdynamic pulmonary circulation. Such data would infor-
m 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 chil-
dren had a history of acute chest syndrome and five were on
hydroxyurea therapy, none were undergoing regular blood
transfusions.

Lung Function, \( F_{NO} \) and Effective Pulmonary Blood Flow

The SCD children compared to the controls had a significa-
cantly lower FEV \(_1\) (\( P < 0.0001\)), VC (\( P = 0.0001\)), \( F_{NO} \) (\( P = 0.0071\)),
FEV \(_1\)/VC (\( P = 0.0031\)), TLC (\( P = 0.0002\)) and SpO\(_2\) (\( P = 0.0023\))
and a significantly higher \( R \) (\( P = 0.0008\)) and \( K NO \) (\( P = 0.0114\))
(Table 1). In the SCD group, three patients (17%) had restric-
tive, 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 reduc-
tion in DLCO.

There were no significant differences between the groups
with regard to \( F_{NO} \) or total maximal airway NO flux
(\( F_{NO} \)) (Table 2). Compared to the control group, the SCD
patients had a significantly higher alveolar NO concentration
(\( C m NO \)) (\( P = 0.0007\)), rate of alveolar NO production (\( N^m NO \))
(\( P = 0.0224\)), effective pulmonary blood flow (\( Q^{EFPB} \)) (\( P < 0.0006\)) and
pulmonary blood flow index (\( Q^{EFPB}_{100} \)) (\( P < 0.0001\)).

| Table 1. Demographics and lung function by sickle cell disease
status |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sickle cell disease</td>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>18</td>
<td>n = 18</td>
<td>P value</td>
</tr>
<tr>
<td>Sex (female m(%))</td>
<td>9 (50%)</td>
<td>9 (50%)</td>
<td>1.0000</td>
</tr>
<tr>
<td>Age (years)</td>
<td>16.0 (18–18)</td>
<td>17.0 (18–18)</td>
<td>0.3130</td>
</tr>
<tr>
<td>(Hb)(g/dl)</td>
<td>8.5 (6.0–10.0)</td>
<td>9.1 (7.8–10.0)</td>
<td></td>
</tr>
<tr>
<td>FEV (_1) (l)</td>
<td>104.5 (76.6–124.0)</td>
<td>110.6 (92.6–124.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>VC (l)</td>
<td>104.8 (86.0–104.6)</td>
<td>102.8 (89.2–102.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>( F_{NO} ) (ppb)</td>
<td>58.3 (39.2–133.0)</td>
<td>92.4 (53.5–153.0)</td>
<td>0.0071</td>
</tr>
<tr>
<td>( F_{NO}/VC )</td>
<td>91.0 (50.2–104.5)</td>
<td>98.8 (95.3–112.3)</td>
<td>0.0315</td>
</tr>
<tr>
<td>TLC (l)</td>
<td>83.7 (73.8–106.5)</td>
<td>102.5 (85.5–125.3)</td>
<td>0.0024</td>
</tr>
<tr>
<td>IR (l)</td>
<td>85.1 (31.9–125.3)</td>
<td>93.0 (58.1–115.3)</td>
<td>0.1961</td>
</tr>
<tr>
<td>DLCO (l/min/mmHg)</td>
<td>88.5 (60.0–122.0)</td>
<td>87.2 (61.4–127.7)</td>
<td>0.5881</td>
</tr>
<tr>
<td>( K NO ) (l/min/mmHg)</td>
<td>91.6 (70.0–132.0)</td>
<td>86.2 (68.2–113.0)</td>
<td>0.0114</td>
</tr>
<tr>
<td>( R ) (l/min/mmHg)</td>
<td>114 (107.8–214.1)</td>
<td>111.7 (92.2–166.4)</td>
<td>0.0008</td>
</tr>
<tr>
<td>SpO(_2) (%)</td>
<td>95 (90–99)</td>
<td>98 (95–99)</td>
<td>0.0023</td>
</tr>
<tr>
<td>Pulse Oximeter</td>
<td>62.7 (57.9–83.8)</td>
<td>85.1 (44.3–113.3)</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

In the SCD children and the controls, no significant correla-
tions were observed between \( F_{NO} \) indices and \( R \) or FEV \(_1\)/VC
(Table 3). In the SCD group, \( Q_{EFPB} \) was positively correlated with
\( F_{NO} \) (\( r = 0.591 \), \( P = 0.0112 \)), \( C m NO \) (\( r = 0.742 \), \( P = 0.0034 \)) and \( V_{m CO} \) (\( r = 0.0002 \))
(Table 4). \( Q_{EFPB} \) was positively correlated with \( F_{NO} \) (\( r = 0.591 \), \( P = 0.0112 \)), \( C m NO \) (\( r = 0.742 \), \( P = 0.0034 \)) and \( V_{m CO} \) (\( r = 0.806 \),
\( P < 0.0001 \)) (Figure 1). No significant correlations were seen
between \( F_{NO} \) indices and \( Q_{EFPB} \) or \( Q_{EFPB}^m \) in the control group
(Table 4). In the SCD children, hemoglobin concentration was
 correlated with \( Q_{PULM} \) (\( r = 0.56 \), \( P = 0.0157 \)) and alveolar NO concentration
(\( r = 0.46 \), \( P = 0.0480 \)).

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

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

<table>
<thead>
<tr>
<th>Sickle cell disease children</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>F_{exp}</td>
<td>0.340</td>
<td>0.1681</td>
</tr>
<tr>
<td>F_{exp}</td>
<td>0.286</td>
<td>0.2493</td>
</tr>
<tr>
<td>F_{exp}</td>
<td>0.161</td>
<td>0.5139</td>
</tr>
<tr>
<td>F_{exp}</td>
<td>0.220</td>
<td>0.3865</td>
</tr>
<tr>
<td>F_{exp}</td>
<td>0.214</td>
<td>0.3931</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F_{exp}</td>
<td>0.311</td>
<td>0.2234</td>
</tr>
<tr>
<td>F_{exp}</td>
<td>0.245</td>
<td>0.3417</td>
</tr>
<tr>
<td>F_{exp}</td>
<td>0.220</td>
<td>0.3865</td>
</tr>
<tr>
<td>F_{exp}</td>
<td>0.251</td>
<td>0.3153</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F_{exp}</td>
<td>0.094</td>
<td>0.7108</td>
</tr>
<tr>
<td>Controls</td>
<td>0.067</td>
<td>0.2834</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Sickle cell disease children</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q_{air}</td>
<td>0.996</td>
<td>0.001</td>
</tr>
<tr>
<td>Q_{air}</td>
<td>0.991</td>
<td>0.001</td>
</tr>
<tr>
<td>Q_{air}</td>
<td>0.528</td>
<td>0.1834</td>
</tr>
<tr>
<td>Q_{air}</td>
<td>0.200</td>
<td>0.2423</td>
</tr>
<tr>
<td>Q_{air}</td>
<td>0.080</td>
<td>0.0043</td>
</tr>
<tr>
<td>Q_{air}</td>
<td>0.342</td>
<td>0.0006</td>
</tr>
<tr>
<td>Q_{air}</td>
<td>0.261</td>
<td>0.0092</td>
</tr>
<tr>
<td>Q_{air}</td>
<td>0.886</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q_{air}</td>
<td>0.930</td>
<td>0.7042</td>
</tr>
<tr>
<td>Q_{air}</td>
<td>0.935</td>
<td>0.6485</td>
</tr>
<tr>
<td>Q_{air}</td>
<td>0.311</td>
<td>0.5631</td>
</tr>
<tr>
<td>Q_{air}</td>
<td>0.1600</td>
<td>0.5193</td>
</tr>
<tr>
<td>Q_{air}</td>
<td>0.1804</td>
<td>0.4885</td>
</tr>
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<td>Q_{air}</td>
<td>0.0233</td>
<td>0.9293</td>
</tr>
<tr>
<td>Q_{air}</td>
<td>0.4256</td>
<td>0.0886</td>
</tr>
<tr>
<td>Q_{air}</td>
<td>0.1936</td>
<td>0.4550</td>
</tr>
</tbody>
</table>

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, Gergis et al. (11) reported a reduction in F_{exp}, 50 ml/min 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_{exp} indices and FRC or FEV/FVC. A larger study (13) reported, as we now do, no difference in F_{exp} when 80 SCD children were compared to 50 ethnic-matched controls. Furthermore, airway obstruction, as evidenced by a reduced FEV/FVC, was not associated with increased methacholine sensitivity or elevated F_{exp} (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 airway obstruction seen in SCD at least in certain patients may reflect increased pulmonary capillary blood volume rather than asthma (9,19).

To the best of our knowledge, this study is the first to assess the relationship between pulmonary hemodynamics, F_{exp} maximal airway NO flux, and alveolar NO concentrations 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_{NO} suggest that the elevated alveolar NO production was associated with a hypertensive circulation in SCD patients. In patients with
Articles

Liver cirrhosis and hepato-pulmonary 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_{alveolar} 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_{alveolar} 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_{alveolar} 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_{NO} 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 arteriography or measurements derived from CT pulmonary angiography. The method and device we used has been previously validated in children (23) 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 that has been present in the SCD children. All those 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 controls, 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 years of the SCD children. Only children over 7 years 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 weeks 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 (approved number 08/H0806/29) 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 50 Hz (R) was measured during a 5-minute period of tidal breathing using impulse oscillometry (IOS, Jaeger MasterScreen IOS, Carepoint, 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_{NO}.

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 (FEV1), VC, mean maximal expiratory flow (FEF_25-75), 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 30 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, Carepoint, Basingstoke, UK). Carboxyhaemoglobin levels were measured to exclude carboxyhaemooglobinemia. If TLC was less than the lower limit of normal with a normal FEV1, VC an obstructive abnormality if their FEV1/VC was less than lower limit of normal and a mixed pattern if both TLC and FEV1/VC were less than the lower limit of normal (34).

DLNO Measurements

Exhaled nitric oxide was assessed according to American Thoracic Society/European Thoracic Society criteria (33). Exhalation was performed at target flows of 50, 100, 150, and 200 mL/s against a final resistance to maintain valve-pharyngeal closure. Respiratory flow was recorded using an integral flow meter during each maneuver and used to calculate NO output. Linear regression of the NO output against flow vs. flow was performed to derive the total maximal alveolar NO flux (F_{NO, max} (nl/min)) and the alveolar NO concentration (C_{NO, alveolar} (ppb)) using the model of Contedrilli et al. (19) which allows for possible contamination of the alveolar region by dead-diffusion of Alveolar from the airway compartment. A commercial online NO analyser system (Hypex FNO, Mediscan Cardiac respiratory instrumentation, Sofram, Belgium) was used for all measurements. Exhaled NO measurements were performed without a noseclip. The equation of Pellec et al. (30) was used to derive the rate of alveolar NO production (Q_{NO, alveolar} (nl/min)) as follows:

\[ V_{NO, alveolar} = DLNO \times C_{NO, alveolar} \times (\text{respired NO mass} - \text{exhaled NO mass}) \]

where F_{NO, max} is the maximum partial pressure (assumed to be 47 mm Hg) and P_{NO, max} is the atmospheric pressure and D_{NO} is expressed in nl/mm Hg. DLNO measurements were performed before the DLNO- DLNO measures 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_{Pulmonary}) was measured noninvasively by the inert-gas rebreathing
method using a commercially available device (Innomax, Innovision Aps, Glamsbjerg, Denmark). The inspired gas mixture contained 0.5% nitrous oxide (N₂O) and 0.15% nitric oxide (NO) as the volatile and insoluble components respectively (35). To adjust for body size, the pulmonary blood flow index (Q₂/TS) was derived by dividing Q₂ by the predicted body surface area using the formula of Mosteller (35).

Analysis

The data were not normally distributed, therefore differences between groups were assessed using the Wilcoxon rank test. Correlations as assessed by Spearman rank coefficient were calculated to determine the strength of relationships between arterial NO flux and airflow obstruction (FEV₁, VC and FEF₂₅₋₇₅) 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

Comparisons 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 0.05 level.

ACKNOWLEDGMENTS

A.J. and A.G. designed the study, A.L. and N.A. collected the data; A.L. and A.G. analyzed the data. All authors were involved in the production of the final manuscript.

STATEMENT OF FINANCIAL SUPPORT

This research was supported by the National Institute for Health Research (NIHR) Biomedical Research Centre at Guy’s and St. Thomas’ NIHR Foundation Trust and King College London. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR, or the Department of Health. Disclosure: None to declare.

REFERENCES

Longitudinal Assessment of Lung Function in Children With Sickle Cell Disease

Alan Lunt, BSc, MD, Karl Sylvester, PhD, Gerard Rafferty, PhD, Moira Dick, BSc, MD, David Rees, MSc, Susan Height, MD, Swee Lay Thein, FRCP, and Anne Greenough, MD

Summary. Objectives. To prospectively assess longitudinal lung function in children with sickle cell disease (SCD). Working hypothesis: Lung function in SCD children deteriorates with increasing age and the decline is more marked in younger children who have recently suffered ACS episodes. Study design. Two prospective longitudinal studies. Patient subject selection. Two cohorts of SCD children and age and ethnic matched controls were recruited. Cohort One (47 SCD and 26 controls) had a median age of 9.8 years and follow up of 2 years and Cohort Two (45 SCD and 26 controls) a median age of 10.2 years and follow up of 10 years. Methodology. Forced expiratory volume in one second (FEV1), vital capacity (VC), forced expiratory flow between 25% and 75% of VC (FEF25-75), total lung capacity (TLC) and residual volume (RV) were measured on two occasions. Results. In both groups of SCD children, lung function declined significantly, but in neither control group. ACS episodes were more frequent during the follow-up period in Cohort One than in Cohort Two (p = 0.0001). The rate of decline was greater in Cohort One than Cohort Two for FEV1 (p = 0.006), VC (p = 0.001), FEF25-75 (p = 0.004), and TLC (p = 0.0044) and RV (p = 0.0433). In Cohort Two restrictive abnormalities were more common at follow up (p = 0.008). Conclusions. Lung function deteriorated with increasing age in SCD children and the rate of decline was greater in younger children in whom ACS episodes were more common.

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Key words. acute chest syndrome; asthma; restrictive lung disease.

Funding source. National Institute for Health Research (NIHR) Biomedical Research Centre, St Thomas’ NHS Foundation Trust and King’s College London.
INTRODUCTION

Sickle cell disease (SCD) is one of the commonest inherited disorders worldwide, affecting an estimated 300,000 newborns every year. The majority of children with SCD in developed countries can expect to survive to adulthood but may then suffer severe respiratory morbidity including chronic hypoxia and pulmonary hypertension. Lung function abnormalities have been reported even in young SCD children, but there is not a consistent picture, cross-sectional studies highlighting restrictive, obstructive, or obstructive abnormalities. One cross-sectional study, however, suggested the occurrence of restrictive abnormalities may increase with increasing age in childhood. More recently, longitudinal studies have been undertaken. In a cohort of 45 children aged between 5 and 18 years measured at baseline and approximately 4 years later, a predominantly obstructive pattern was reported which increased in prevalence over time; the occurrence of restrictive abnormalities also increased, but to a lesser extent. In contrast, retrospective analysis of a larger cohort of 443 SCD children aged between 8 and 18 years, who were measured on two separate occasions with a variable time to follow-up, demonstrated an increased prevalence of restrictive abnormalities with increasing age. There was a decline in total lung capacity (TLC) of 2.3% per year and a similar decline in forced expiratory volume in one second (FEV₁), vital capacity (VC), and forced expiratory flow between 25% and 75% of VC (FEF₂5–75). Neither study included a control group and thus it is not possible to determine whether the decline in lung function reported was only seen in SCD patients and hence the magnitude of the effect of SCD. In addition, it is important to assess whether any difference was influenced by increasing age.

In adults, acute chest syndrome (ACS) is the commonest cause of death and is associated with more severe restrictive disease. In children, ACS episodes have been associated with reductions in lung function. The impact of ACS episodes on longitudinal changes in lung function, however, has not been investigated. There is evidence that asthma may be a risk factor for ACS episodes. Hence, in any subsequent longitudinal study the impact of asthma also needs to be considered.

The aim of this prospective study was to examine the rate of decline in lung function in children with SCD in comparison to age and ethnic matched controls. Two cohorts were studied. Cohort One was younger children who were followed for approximately 2 years, during which time ACS episodes were more common. Cohort Two were older and followed for approximately 10 years. We included two cohorts as comparison of their results would allow us to test our primary hypothesis that the rate of decline in lung function would be greater in the younger cohort as it would relate to their higher occurrence of ACS episodes. Furthermore, in Cohort Two, we wished additionally to test the hypothesis that any obstructive abnormalities demonstrated at the initial assessment would be replaced by restrictive abnormalities at the subsequent assessment. As asthma may be a risk factor for ACS episodes we also wished to determine whether asthma and hence baseline obstructive lung function abnormalities influenced any change in lung function and whether obstructive lung function abnormalities were associated with an increased risk of ACS episodes. Ethnic and age matched controls were recruited for both cohorts to determine whether any decline in lung function was only seen in SCD patients.

MATERIALS AND METHODS

Study Design

A prospective longitudinal study of children with SCD (homozygous for sickle cell hemoglobin (HbSS)) of African-Caribbean or West African descent was undertaken. Age and ethnic matched children without HbSS were recruited as controls; they were siblings of the SCD children or recruited from local schools. Two cohorts were tested, each on two occasions. In Cohort One, changes in lung function over a median follow-up of approximately 2 years and in Cohort Two changes in lung function of children over a median follow-up of 10 years were determined. Cohort Two were recruited earlier during a previous period of research grant funding and both cohorts were followed during a recent period of grant funding. During this period, routine assessment of lung function of SCD patients was not undertaken. Only patients who successfully performed both spirometry and body plethysmography were included in the analysis. The study was approved by the King's College Hospital Research Ethics Committee and parents gave informed written consent for their child to take part.

Lung Function Assessments

Patients were assessed in the Amanda Smith Pulmonary Function Laboratory at King's College Hospital NHS Foundation Trust. No subject underwent lung function testing within 2 weeks of an upper respiratory tract infection or 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 (Holstein Ltd, Croydon, Dyfed, UK) and weight using electronic weighing scales (Seca Ltd, Birmingham, UK).

Spirometry was performed and static lung volumes were assessed using whole-body plethysmography according to
American Thoracic Society/European Thoracic Society criteria. Forced expiratory volume in 1 sec (FEV₁), vital capacity (VC), forced expiratory flow between 25% and 75% of VC (FEF25-75), total lung capacity (TLC), and residual volume (RV) were recorded and the results expressed as the percent predicted for height, age, and sex. The highest forced vital capacity result obtained from spirometry and the slow vital capacity from the "plethysmography" manoeuvre was reported as VC. Subjects were assessed wearing a nose clip and breathing through a mouth piece. Measurements were performed using a pneumotachograph based system (Jaeger Master screen PFT, Carefusion Ltd, Basingstoke UK). Spirometry was repeated following administration of a bronchodilator (400 µg salbutamol) via a MDI and spacer and a positive response was defined as an increase in FEV₁ of greater than or equal to 12% from baseline. Results were expressed as percent predicted for height, age, and sex using the ethnic-specific reference equations for spirometry and the European Community for Steel and Coal Statement of the European Respiratory Society reference equations for lung volumes and gas transfer for patients over 18 years of age and those of Rosenthal et al. for children under eighteen. The predicted values for total lung capacity were reduced by 12% and residual volume by 7% to correct for ethnicity. The lower and upper limits of normal were defined as the fifth and ninety-fifth percentiles respectively of the appropriate reference range. Patients were diagnosed with a restrictive abnormality if their TLC was less than the lower limit of normal (LNN) with a normal FEV₁/VC, an obstructive abnormality if their FEV₁/VC was less than LNN and a mixed pattern if both TLC and FEV₁/VC were less than the LNN.

Clinical and Laboratory Characteristics

The medical records for all SCD children were examined. The occurrence and number of ACS episodes during the study period was recorded for each SCD child. An ACS episode was diagnosed if the child had suffered chest pain, dyspnea, and prexemia together with a new pulmonary infiltrate on chest radiograph. SCD children and controls were diagnosed as having asthma if they were currently prescribed any anti-asthma medications. Whether the child was on a chronic transfusion program or receiving hydroxyurea was also noted. Hemoglobin concentrations for the SCD children were obtained from routine clinical blood samples taken within 2 months of lung function testing and when the patient was clinically stable.

Statistical Analysis

Differences in lung function results at baseline and follow-up were assessed for statistical significance using the Wilcoxon signed-rank test, Fisher’s exact test or Chi squared test as appropriate. Individual lung function results were reported as the percentage predicted in order to normalize results for stature and where appropriate age. Linear mixed model (LMM) analysis was then used to analyze trajectories of decline in lung function in the SCD children relative to the control groups and to determine the influence of asthma, airways obstruction at baseline and the occurrence of ACS during the follow up period. ACS, airways obstruction at baseline and diagnosis of asthma were coded as nominal variables (0 = absent; 1 = present). Models were also fitted to compare the trajectories in the two cohorts. LMM analysis is an extension of multiple regression, which allows irregularly spaced serial data for individuals to be combined in a single linear regression model, providing estimates of both individual changes over time and group patterns. Fixed effects in the models were: ACS during the study period, diagnosis of asthma, obstruction at baseline, age at measurement, and an intercept term. Random effect variables comprised age at measurement and an intercept term. Models were estimated using restricted maximum likelihood (REML), and separate models were fitted for each of the lung function results with all models grand mean centred on age at baseline such that the intercept terms would be interpretable as the mean value of the result at baseline, and the slope would represent the annual rate of change. Interaction terms were introduced to test the equality of slopes between groups. Initial models included all variables and interactions. The validity of including the random effects, and the selection of an appropriate covariance structure for the residuals was assessed by likelihood ratio testing based on the log likelihood difference between the original and modified models. Models were then reduced by the sequential removal of non-significant terms. Likelihood ratio tests based on the difference in −2 log likelihood for initial and reduced models were used to determine whether a non-significant term should be removed. Baseline predictors of future ACS were determined using binary logistic regression.

RESULTS

Demographics

Cohort One: Forty-seven children with SCD and twenty-six controls were assessed; the two groups were of similar age and sex distribution (Table 1). Ten SCD children (21%) had had at least one ACS episode during the study. Nine SCD children and two controls had a diagnosis of asthma (P = 0.908). Seven SCD children (14.9%) were prescribed hydroxyurea and one (2.1%) was on a chronic transfusion programme within the study.

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<table>
<thead>
<tr>
<th>TABLE 1—Demographics by SCD Status</th>
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<tbody>
<tr>
<td>SCD</td>
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<td>-----</td>
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<tr>
<td>Cohort One</td>
</tr>
<tr>
<td>N</td>
</tr>
<tr>
<td>Males (%)</td>
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<tr>
<td>Age at Baseline (years)</td>
</tr>
<tr>
<td>Time to follow-up (years)</td>
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<tr>
<td>Hb (g/dL)</td>
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<tr>
<td>Cohort Two</td>
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<tr>
<td>N</td>
</tr>
<tr>
<td>Males (%)</td>
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<tr>
<td>Age at Baseline (years)</td>
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<tr>
<td>Time to follow-up (years)</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
</tr>
</tbody>
</table>

Data are demonstrated as n (%) or median (range).

Lung Function Abnormalities at Baseline and Follow-Up

At baseline, sixteen SCD children in Cohort One (34%) had an obstructive pattern, and one (2%) a restrictive defect; no child had a mixed defect. At follow-up, the number of subjects with an obstructive defect had declined to five (10.6%, P = 0.021), the number with restrictive defects increased to eight (17%, P = 0.034) and two patients had a mixed pattern (4.3%, P = 0.495). A positive response to a bronchodilator was seen in four SCD children (16.7%) at baseline and three (12.5%) at follow up (P = 1.000). In Cohort Two, at baseline, eleven SCD children (24%) had an obstructive pattern and five (11%) a restrictive defect; no child had a mixed defect. At follow-up, five subjects had an obstructive defect (11.1%, P = 0.167), the number with restrictive defects had increased to twenty (44%, P = 0.0008) and three patients had a mixed pattern (6.7%, P = 0.242). A positive bronchodilator response was seen in seven subjects (15.6%) at baseline and one subject (2.2%) at follow-up (P = 0.059).

At baseline, the proportion of SCD children with obstructive and restrictive defects was similar in both cohorts (P = 0.364, P = 0.107 respectively). At follow-up, Cohort Two compared to Cohort One had a greater proportion of SCD children with a restrictive defect (P = 0.006), but a similar number with an obstructive defect (P = 0.792). In Cohort Two, patients with an obstructive defect at baseline were more likely to have a restrictive abnormality at follow-up (P = 0.0289).

In neither control group was there a significant change in the patterns of lung function over the follow-up period. Amongst the controls in Cohort One, three had an obstructive abnormality and none a restrictive abnormality.

<table>
<thead>
<tr>
<th>TABLE 2—Lung Function in Cohort One by Follow-Up Status</th>
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<tbody>
<tr>
<td>Baseline</td>
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<tr>
<td>----------</td>
</tr>
<tr>
<td>SCD children</td>
</tr>
<tr>
<td>FEV1</td>
</tr>
<tr>
<td>VC</td>
</tr>
<tr>
<td>FEF25-75</td>
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<tr>
<td>FEV1/VC</td>
</tr>
<tr>
<td>TLC</td>
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<tr>
<td>RV</td>
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<tr>
<td>FV TLC</td>
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<tr>
<td>Controls</td>
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<tr>
<td>FEV1</td>
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<tr>
<td>VC</td>
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<tr>
<td>FEF25-75</td>
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<tr>
<td>FEV1/VC</td>
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<tr>
<td>TLC</td>
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<tr>
<td>RV</td>
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<tr>
<td>FV TLC</td>
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</tbody>
</table>

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### TABLE 3—Lung Function in Cohort Two by Follow Up Status

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Follow-up</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCD children</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV₁</td>
<td>90.7</td>
<td>81.2</td>
<td>0.0002</td>
</tr>
<tr>
<td>VC</td>
<td>97.0</td>
<td>85.4</td>
<td>0.0003</td>
</tr>
<tr>
<td>FEV₁/VC</td>
<td>91.8</td>
<td>74.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>TLC</td>
<td>96.2</td>
<td>89.4</td>
<td>0.7668</td>
</tr>
<tr>
<td>RV</td>
<td>92.5</td>
<td>81.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TLC/RV</td>
<td>102.2</td>
<td>88.9</td>
<td>0.0389</td>
</tr>
<tr>
<td>Controls</td>
<td>121.2</td>
<td>113.2</td>
<td>0.0092</td>
</tr>
</tbody>
</table>

#### Control

- **FEV₁**: 99.8 (79.3–109.8) vs. 100.3 (78.6–114.7), \( P = 0.0468 \)
- **VC**: 103.1 (71.3–124.1) vs. 107.9 (76.2–124.6), \( P = 0.1747 \)
- **FEV₁/VC**: 92.7 (55.3–163.6) vs. 102.3 (54.7–163.0), \( P = 0.0891 \)
- **FEV₁/VC**: 98.3 (61.1–112.2) vs. 96.3 (48.9–116.6), \( P = 0.7425 \)
- **TLC**: 98.3 (78.5–111.8) vs. 94.0 (78.4–121.8), \( P = 0.9421 \)
- **RV**: 95.7 (80.3–146.1) vs. 91.1 (48.0–154.3), \( P = 0.9381 \)
- **TLC/RV**: 110.2 (63.2–175.7) vs. 94.6 (45.6–158.7), \( P = 0.0742 \)

Data are expressed as percent predicted and presented as median (range).

at baseline and follow up. Among the controls in Cohort Two, two had an obstructive lung function abnormality and none a restrictive abnormality at baseline and follow up.

### Lung Function Abnormalities and ACS

In both cohorts, there were significant correlations in the SCD children between obstructive defects at baseline and ACS episodes (\( P = 0.0003 \) for Cohort One, \( P = 0.028 \) for Cohort Two). In Cohorts One and Two, in the SCD children the presence of obstructive defects at baseline was predictive of an ACS episode during the study: odds ratio (OR) 13.9 (95% CI 2.5–77.0), \( P = 0.003 \) and 5.4 (95% CI 1.2–23.7), \( P = 0.026 \) respectively. In Cohort One there was no independent association in the SCD children between asthma and a subsequent ACS episode: OR 6.1 (95% CI 0.5–12.8, \( P = 0.070 \)). ACS episodes occurred more frequently in the SCD children in Cohort One than Cohort Two (one episode per 1.93 patient/years vs. one episode per 12.6 patient/years, \( P < 0.0001 \)).

### Lung Function Decline in SCD Children and Controls

In Cohort One, a significant decline was observed in the SCD children relative to the controls in FEV₁ (\( P = 0.027 \)), VC (\( P < 0.0001 \), FEV₁/VC (\( P = 0.042 \)), TLC (\( P = 0.0001 \)) and RV (\( P = 0.001 \)) (see Supplement Table S3). In Cohort Two, a significant decline was observed relative to the controls in FEV₁ (\( P = 0.001 \), VC (\( P = 0.004 \), FEV₁/VC (\( P = 0.016 \)) and TLC (\( P = 0.002 \)) (see Supplement Table S4). The rate of decline was significantly greater in Cohort One than Cohort Two for FEV₁ (\( P = 0.008 \)), VC (\( P = 0.001 \), FEV₁/VC (\( P = 0.030 \)), TLC (\( P = 0.004 \)), and RV (\( P = 0.043 \)) (see Supplement Table S5). No significant effects of hydroxyurea use or chronic transfusion were seen during exploratory analysis when those factors were included as covariates in the models (see Supplement Tables S6 and 7).

No significant effects of hydroxyurea use or chronic transfusion were seen during exploratory analysis when those factors were included as covariates in the models (see Supplement Tables S6 and 7).

**DISCUSSION**

We have demonstrated that lung function declines in children with SCD compared to similar aged, ethnic matched controls. The average annual rate of decline for all lung volumes was significantly greater in Cohort One compared to Cohort Two, which suggests the most rapid period of deterioration takes place during early childhood. This deterioration represents a progression towards a restrictive pattern of lung function with increasing age.

We demonstrated that ACS episodes were the only independent predictor of a greater decline in lung volumes. ACS episodes occurred more frequently per unit follow up period in Cohort One who were followed up for 2 years compared to Cohort Two who were followed up for 10 years. Thus ACS episodes might explain the greater rate of decline in lung function in Cohort One and hence our results emphasize the need for more effective methods of preventing ACS episodes, such as an increased use of hydroxyurea.

The rate of decline in lung volumes observed in the SCD children in Cohort One, that is the younger children, was similar to that demonstrated by MacLean et al. who observed an annual reduction in TLC of around 2.3% per year, with proportionate reductions in FEV₁, VC, and TLC. Unlike MacLean et al., however, we did not detect a decline in RV/TLC in the SCD children. This difference may be explained by MacLean not expressing RV/TLC as a percentage of the predicted value and therefore not accounting for age-related changes in RV/TLC.

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The prevalence of obstructive lung disease in the SCD children at baseline was similar in both cohorts (34% and 24% respectively) and to that observed by Koumoutsiris et al. (22%). However, but higher than that reported by Maclean et al. The latter difference may be due to Maclean’s use of a fixed cutoff for FEV1/VC to define an obstructive defect, whereas this study used the lower limit of normal based on a reference range. After criticism from Koumoutsiris, Maclean et al. subsequently reanalyzed their data using the lower limit of normal to define abnormality and found that more patients had a diagnosis of obstruction than reported in the original study. The proportion of patients with restrictive disease at baseline was lower in the SCD children Cohort One to that observed by Koumoutsiris (22%), but similar in Cohort Two. Cohort One was, however, younger than the cohort of Koumoutsiris (8.8 vs. 10.6 years), whereas Cohort Two was of a similar age (10.2 years). The prevalence of restrictive defects at baseline was higher at follow-up in Cohort Two than in Maclean’s cohort when assessed at age seventeen (18.7%). That difference may be explained by Maclean using a fixed cutoff of <70% predicted for TLC to define restriction, which may have resulted in mis-diagnosis.

Although, ACS episodes were linked to a faster progression of restrictive disease, whether the prompt treatment of airflow obstruction in SCD might help prevent the development of restrictive lung disease in later life is unknown. The incidence of obstructive defects at baseline was higher than that of a diagnosis of asthma, which suggests other mechanisms may be responsible for obstructive defects in SCD. Indeed, we have recently demonstrated an association between pulmonary vascular engorgement and obstructive lung function in both adults and children with SCD. Hence, any preventative strategy needs to consider this abnormality in SCD children.

This study has strengths and some limitations. The novelty of our data is that we have longitudinally assessed SCD patients and ethnic and age matched controls. To the best of our knowledge, this is the first longitudinal study to include matched controls evaluated in parallel with SCD children. We also monitored patients prospectively and used a consistent definition to document the occurrence of ACS episodes during the study period. The inclusion of two SCD groups with two and 10-year follow-up periods respectively allowed both the short and long-term changes in lung function in childhood to be evaluated. We used ethnic-specific reference equations for spirometric results, but the static lung volume results were related to two reference ranges derived from Caucasian children and adults with a fixed correction factor to account for ethnicity as no ethnic-specific reference values are currently available. This adjustment is, however, imprecise and may have resulted in some inaccuracy. The change in lung function was, therefore, referenced to an ethnic matched control group in each cohort so that interpretation of within-cohort changes in the SCD children was valid. Indeed, the pattern of change in static lung volumes was similar to that in spirometric measurements. We did not exclude patients who were treated with hydroxyurea or chronic transfusions, as this would have biased the study population in favor of SCD children with less severe disease. Inclusion of these treatments into the analysis did not result in any significant differences in the results, but the number of children receiving such treatments was small and hence it would be inappropriate to draw conclusions from these results.

In conclusion, lung function in children with sickle cell disease declines and the rate of decline was greater in young children in whom ACS episodes were more common. Our results suggest treatment strategies to prevent ACS episodes need to be started in young SCD children if they are to be most effective in preventing the decline in lung function.

AUTHORS’ CONTRIBUTIONS

AG and AL designed the study, AL, KS, and EMcG collected the data and AL and AG analyzed the data. All authors were involved in the production of the manuscript.

REFERENCES

Lung Function in SCD Children


SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher’s web-site.
10.5. Airways Obstruction and Pulmonary Capillary Blood Volume in Children With Sickle Cell Disease

Airways Obstruction and Pulmonary Capillary Blood Volume in Children With Sickle Cell Disease

Catherine J Wedderburn, MBChB, David Rees, FRCP, Susan Height, MD, Moira Dick, MB, BCH, BAO, Gerrard F Rafferty, PhD, Alan Lunt, BSc (Hons), and Anne Greenough, MD

Summary. Objectives and Working Hypothesis: Airways obstruction occurs in young children with sickle cell disease (SCD). Our aim was to test the hypothesis that increased pulmonary capillary blood volume at least in part explained the increased airways obstruction as this would inform which therapy might be most appropriate to treat the airways obstruction. Study design: Observational study. Patients/subject selection: Twenty-five SCD children and 25 ethnic origin matched controls were recruited. Methodology: Respiratory system compliance, using impulse oscillometry at 5 Hz (RC5 'tipped'), pulmonary capillary blood volume (Vo), and spirometry were assessed before and after bronchodilator (ipratropium bromide). Lung volume measurements were also made. Results: The SCD children compared to the controls had a higher RC5 'tipped' before (median 133 (range 88–161)% vs 102 (63–184)%; P = 0.0046) and after (126 (79–150)% vs 91 (46–145)%; P = 0.0448) bronchodilator and their median Vo/VA (ml/L) was higher before (0.028 (0.009) vs. 0.014 (0.008); P < 0.0001) and after (0.025 (0.009) vs. 0.019 (0.008); P < 0.0001) bronchodilator. There were similar decreases in RC5 'tipped' post-bronchodilator in the two groups, but no significant changes in Vo/VA in either group. Vo/VA correlated significantly with RC5 'tipped' in the SCD children only. Conclusions: Increased pulmonary capillary blood volume contributes to the increased airways obstruction in children with SCD. Hence, bronchodilators may be of limited benefit in reducing their airways obstruction.

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Key words: anemia; impulse oscillometry; lung function.

Funding source: National Institute for Health Research (NIHR) Biomedical Research Centre

INTRODUCTION

Sickle cell disease (SCD) is one of the most prevalent inherited disorders worldwide; it is estimated that approximately 250,000 children are born with homozygous SCD per year. Obstructive lung function abnormalities occur in young children with SCD. It is important to understand the etiology of such abnormalities to better inform therapy for children with SCD.

A high prevalence of asthma in children with SCD has been reported in one study. The response to bronchial challenges, such as cold air, exercise, or methacholine, however, has been variable ranging from a positive response rate of 14% to 78%. In addition, whereas some studies have shown a high response rate to bronchodilators in SCD children compared to controls, others have shown no significant difference in the response rates of SCD children and controls. Dilation of the pulmonary vasculature resulting from the increased pulmonary capillary blood volume, which occurs in SCD patients as a result of chronic anemia may increase peripheral airway obstruction. Inflation of the leg compartments of pneumatic trousers increases thoracic blood volume and increased respiratory resistance in healthy volunteers. Acute intravascular volume expansion increased air flow obstruction in patients with left ventricular dysfunction. In addition, in patients with impaired left ventricular function, pre-treatment with...
methoxamine a potent vasoconstrictor, fully prevented the methacholine induced decrease in forced expiratory volume in one second (FEV₁) suggesting that the broncho-constriction was mediated, at least in part, by dilatation of the pulmonary vessels.11 The aim of this study was to determine whether in children with SCD their increased pulmonary capillary blood volume was associated with peripheral airways obstruction, as this would inform which therapy was most appropriate to treat airways obstruction. We, therefore, determined whether there were significant correlations between respiratory system resistance and pulmonary capillary blood volume before and/or after bronchodilator administration in SCD and age and ethnic matched controls. A positive correlation of the respiratory system resistance and pulmonary capillary blood volume results in the SCD children, but not the controls, would suggest their airways obstruction was at least in part related to their increased pulmonary blood volume.

MATERIALS AND METHODS

Study Design

Afro-Caribbean children with SCD (homozygous for sickle cell hemoglobin (HbSS)) were recruited. Age and ethnic matched children without HbSS were recruited as controls, they were siblings of the SCD children or from local schools. Only children over 7 years of age were recruited as they were likely to be able to complete the lung function tests. Peripheral airways resistance, spirometry and then pulmonary capillary blood volume, were assessed before and 30 min after bronchodilator (ipratropium bromide) administration. Lung volume was only assessed prior to bronchodilator administration. An anticholinergic, ipratropium bromide, was used as the bronchodilator as it has minimal cardiovascular side effects. Each subject was given eight puffs of ipratropium bromide via a metered dose inhaler and spacer device (20 µg per metered inhalation, i.e., total dose 160 µg). We assessed the children pre and post bronchodilator to determine whether any changes were greater in the SCD children. In addition, we assessed the children using spirometry and by measuring their lung volumes to further characterize any lung function abnormalities. The study was approved by the King’s College Hospital Research Ethics Committee and parents gave informed written consent for their child to take part.

Lung Function

The study was conducted in the Amanda Smith Unit, King’s College Hospital. No child underwent lung function testing within 2 weeks 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 Ltd, Crymych, Dyfed, UK), and weight using electronic weighing scales (Seca ltd, Birmingham, UK).

Subjects were assessed wearing a nose-clip and breathing through a mouthpiece. Respiratory system resistance was measured during a 90 sec period of tidal breathing using impulse oscillometry (Jaeger Master screen IOS, Carefusion Ltd, Basingstoke UK). As the majority of the pulmonary blood volume is in the periphery of the lung,12 a resistance at 5 Hz was used, as low oscillation frequencies have been shown to be transmitted more distally in the lung and therefore may reflect changes in peripheral airway calibre,13 which could be influenced by an increased pulmonary capillary blood volume. The results were expressed as the percent predicted for height (R5 %pred).14,15 The mean of two measurements with 5% of each other was reported. We diagnosed the children to have an obstructive abnormality if the R5 results were greater than the mean ± 2 standard deviations of the results of our control group. A significant response to bronchodilator was a decrease in R5 >1 standard deviation16 based on the reference data of Nowowiejska et al.17

Spirometry and static lung volumes using whole body plethysmography were then assessed according to American Thoracic Society/European Thoracic Society criteria.16,17 Forced expiratory volume in 1 sec (FEV₁), vital capacity (VC), mean maximum inspiratory flow (MMIF 25-75), total lung capacity (TLC), and residual volume (RV) were recorded and the results expressed as the percent predicted for height.16-18 Measurements were performed using a pneumotachograph based system (Jaeger MasterScreen PFT, Carefusion Ltd, Basingstoke UK). Patients were further considered to have obstructive abnormalities if their FEV₁/VC was less than the lower limit of normal, identified as a z-score ≤ -1.64 based on the ethnic specific reference range of Quanjer et al.15 Lower airways obstruction was diagnosed if the MMIF25/75 was below the lower limit of normal defined as a z-score ≤ -1.64 based on the ethnic specific reference range of Quanjer et al.15 in the absence of a reduced TLC or VC. A restrictive abnormality was diagnosed if the TLC was below the lower limit of normal defined as a z-score ≤ -1.64 based on the reference range of Rosenthal et al.20 with a −12% correction factor applied.21

Pulmonary Capillary Blood Volume

The single breath hold method for gas transfer for carbon monoxide (DLCO) and nitric oxide (DLNO) was used (Jaeger MasterScreen PFT Pro Carefusion Ltd, Basingstoke, UK). Pulmonary membrane diffusing capacity (DMCO) and pulmonary capillary blood volume
DMCO is the carbon conductance across the alveolar-capillary tissue membrane and plasma barrier. $6\text{CO}$ is the rate of carbon monoxide uptake by the whole blood and combination with hemoglobin and $V_c$ is the pulmonary capillary blood volume. Lung diffusing capacity may also be estimated simultaneously using nitric oxide (NO) as the tracer gas (DLNO). The reaction rate of NO binding to hemoglobin is very much greater than that of carbon monoxide, therefore, the rate of NO uptake by blood ($6\text{NO}$) is extremely large and $1/6\text{CO} \cdot V_c$ becomes very small and may be neglected. Therefore, $DLNODMNO = cDMCO$. Where the coefficient $\alpha$ describes the relationship between DLNO and DLCO and from Graham’s law is equal to 1.97. This is then substituted into the Roughton-Forster equation which may be rearranged to yield $V_c$.25

The subject was asked to inspire maximally, maximally expire and then make a second maximal inspiration of the test gas (0.28% carbon monoxide, 9% helium, 19% oxygen, and 40 ppm nitrogen). The subject then breath-held for 8 sec26 after which they had reached maximal inspiration. At the end of the maximal inspiration, a shutter closed to block the “airway” for the 8 sec, after which the subject exhaled. If the subject inspired to <90% of their maximum vital capacity, as determined from their spirometry results, the measurement was discarded and the manoeuvre repeated. The first 750 ml of expired gas was discarded and the next 750 ml was collected for analysis. Pulmonary capillary blood volume was expressed as $V_c$ per unit alveolar volume (Vc/Va). The mean of two measurements within 5% was reported. Results were corrected for hemoglobin concentration (Hb). For the SCD children, the Hb, reticulocyte and lactate dehydrogenase (LDH) results were obtained from blood tests within 1 month of testing. For the control subjects, Hb was measured non-invasively using Hb-pulse oximetry with a pediatric probe (Maximo Radical 7 = rainbow probe, Maximo, CA).

Analysis

The data were not normally distributed, therefore differences were assessed using the Wilcoxon rank test or Mann–Whitney U test as appropriate. Spearman’s correlations were calculated to determine the strength of relationships. Statistical analysis was performed using Prism software (Graphpad Software Inc, San Diego, CA).

TABLE I—Demographics by SCD Status

<table>
<thead>
<tr>
<th></th>
<th>SCD children</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>13.4 (7.4–18.2)</td>
<td>13.0 (7.4–18.0)</td>
<td>0.4318</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>11 (44)</td>
<td>8 (32)</td>
<td>0.5607</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>8.2 (6.1–10.3)</td>
<td>12.5 (10.1–15.0)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

The results are expressed as n (%) or median (range).

Pediatric Pulmonology
TABLE 2—Perihedral Airways Resistance and Pulmonary Capillary Blood Volume Pre- and Post-Bronchodilator According to SCD Status

<table>
<thead>
<tr>
<th></th>
<th>SCD children</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Pre-bronchodilator:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R5 Spid</td>
<td>132.7 (87.7–181.1)</td>
<td>102.4 (83.3–184.2)</td>
<td>0.0046</td>
</tr>
<tr>
<td>Vc/Va (ml/ko)</td>
<td>25.7 (18.2–38.4)</td>
<td>18.4 (13.9–25.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Post-bronchodilator:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R5 Spid</td>
<td>105.0 (78.7–150.0)</td>
<td>91.3 (64.3–147.4)</td>
<td>0.049</td>
</tr>
<tr>
<td>Vc/Va (ml/ko)</td>
<td>25.8 (18.5–41.9)</td>
<td>17.7 (13.1–27.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Change in R5 Spid post-bronchodilator (%)</td>
<td>–18.5 (–36.0 to –4.0)</td>
<td>–12.5 (–29.9 to –5.6)</td>
<td>0.090</td>
</tr>
<tr>
<td>Change in Vc/Va post-bronchodilator (%)</td>
<td>–1.6 (–8.4–6.1)</td>
<td>–2.4 (–6.3–2.0)</td>
<td>0.587</td>
</tr>
</tbody>
</table>

R5 Spid, impulse oscillometry at 5 Hz and expressed as the percentage predicted for height; Vc/Va, pulmonary capillary blood volume/ml/kg volume.

The results are expressed as median (range).

FEV1/VC following bronchodilator administration (Table 4). There were, however, no significant differences in the magnitude of change in lung function following bronchodilator administration between the two groups.

Seven SCD children had an obstructive abnormality (three had only lower airways obstruction) compared to two in the control group (P = 0.1383) based on their spirometry results. Five SCD children, but no controls had a restrictive abnormality. Six SCD children and two controls had an elevated R5 %pred. All six SCD children and one of the two controls with an elevated R5 %pred had an MMEF25/75 below the lower limit of normal. The SCD children with an elevated R5 %pred result compared to those who did not had higher Vc/Va results (median 32.0 (range 23.9–37.7) ml/ko vs. median 25.3 (range 18.2–38.35) ml/ko, P = 0.0116). Four of the six children with elevated R5 %pred and the two controls with elevated R5 %pred had a significant response to bronchodilator. All four SCD patients who had a significant decrease in R5 %pred post bronchodilator had a significant increase in MMEF25/75.

There was a significant correlation in the children overall between R5 %pred and pulmonary capillary blood volume (Fig. 1). In the SCD children, there were significant correlations between R5 %pred and Vc/Va both pre-bronchodilator (r = 0.540, P = 0.005) and post-bronchodilator (r = 0.482, P = 0.015). The R5 %pred in the SCD children both pre (r = −0.453, P = 0.023) and post (r = −0.400, P = 0.048) bronchodilator correlated with the hemoglobin levels, but not the reticulocyte counts (P = 0.633) or the LDH levels (P = 0.556). In the controls, there were no significant correlations between R5 %pred and Vc/Va either pre-bronchodilator (P = 0.319) or post bronchodilator.

TABLE 3—Spirometry and Lung Volume Data by SCD Status

<table>
<thead>
<tr>
<th></th>
<th>SCD children (n = 25)</th>
<th>Controls (n = 25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-bronchodilator:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV1</td>
<td>84.8 (66.8–125.9)</td>
<td>106.1 (73.4–131.9)</td>
<td>0.0002</td>
</tr>
<tr>
<td>VC</td>
<td>89.8 (87.3–132.9)</td>
<td>106.5 (83.4–140.9)</td>
<td>0.0005</td>
</tr>
<tr>
<td>FEV1/VC</td>
<td>95.8 (72.2–110.8)</td>
<td>90.3 (75.7–120.4)</td>
<td>0.0014</td>
</tr>
<tr>
<td>FEF25–75</td>
<td>89.2 (86.1–138.9)</td>
<td>93.1 (80.1–139.1)</td>
<td>0.2079</td>
</tr>
<tr>
<td>MMEF25/75</td>
<td>71.1 (161.1–120.4)</td>
<td>103.7 (97.5–161.2)</td>
<td>0.0006</td>
</tr>
<tr>
<td>TLC</td>
<td>87.5 (68.5–106.2)</td>
<td>90.2 (79.4–107.8)</td>
<td>0.0120</td>
</tr>
<tr>
<td>RV</td>
<td>87.5 (64.5–143.7)</td>
<td>87.3 (54.6–135.6)</td>
<td>0.2336</td>
</tr>
<tr>
<td>RV/TLC</td>
<td>115.8 (82.3–179.4)</td>
<td>105.1 (69.3–131.5)</td>
<td>0.0228</td>
</tr>
<tr>
<td>FRC</td>
<td>77.9 (56.1–115.1)</td>
<td>81.4 (63.1–101.2)</td>
<td>0.3498</td>
</tr>
<tr>
<td>DLCO</td>
<td>83.6 (87.3–118.8)</td>
<td>81.4 (67.5–111.1)</td>
<td>0.5618</td>
</tr>
<tr>
<td>Post-bronchodilator:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV1</td>
<td>87.8 (68.1–131.2)</td>
<td>108.7 (80.7–133.9)</td>
<td>0.0002</td>
</tr>
<tr>
<td>VC</td>
<td>92.6 (71.4–136.3)</td>
<td>106.6 (83.7–141.7)</td>
<td>0.0021</td>
</tr>
<tr>
<td>FEV1/VC</td>
<td>97.5 (85.8–108.9)</td>
<td>100.6 (80.7–111.2)</td>
<td>0.0328</td>
</tr>
<tr>
<td>FEF25-75</td>
<td>98.5 (98.7–175.6)</td>
<td>100.0 (67.3–130.7)</td>
<td>0.6780</td>
</tr>
<tr>
<td>MMEF25/75</td>
<td>86.5 (81.4–135.0)</td>
<td>116.4 (55.2–171.1)</td>
<td>0.0011</td>
</tr>
</tbody>
</table>

FEV1, forced expiratory volume in 1 sec; VC, vital capacity; FEV1/VC, forced expiratory volume in 1 sec/vital capacity; FEF25-75, peak expiratory flow; MMEF25/75, mean maximum expiratory flow; TLC, total lung capacity; RV, residual volume; RV/TLC, residual volume/total lung capacity; FRC, functional residual capacity.

The results are expressed as median (range). Data are presented as the percentage predicted for height.

Pediatric Pulmonology
TABLE 4—Lung Function Results Related to Bronchodilator Administration

<table>
<thead>
<tr>
<th></th>
<th>Pre-bronchodilator (n = 25)</th>
<th>Post-bronchodilator (n = 25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCD children</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV1</td>
<td>84.8 (66.4–125.9)</td>
<td>87.8 (68.1–131.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MMFR25/75</td>
<td>71.1 (51.1–109.4)</td>
<td>86.2 (58.4–135.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RV/VC</td>
<td>97.3 (65.8–138.9)</td>
<td>97.3 (65.8–138.9)</td>
<td>0.0071</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV1</td>
<td>106.1 (75.4–131.9)</td>
<td>100.7 (80.7–135.9)</td>
<td>0.0002</td>
</tr>
<tr>
<td>MMFR25/75</td>
<td>109.7 (75.4–148.2)</td>
<td>106.4 (55.5–173.1)</td>
<td>0.0011</td>
</tr>
<tr>
<td>FEV1/VC</td>
<td>99.3 (57.5–129.4)</td>
<td>100.6 (60.7–111.2)</td>
<td>0.0128</td>
</tr>
</tbody>
</table>

FEV1, forced expiratory volume in 1 sec; MMFR25/75, mean maximum expiratory flow; FEV1/VC, forced expiratory volume in 1 sec: vital capacity.

The results are expressed as median (range) and as the percentage predicted for height and analyzed by paired analyses.

(P = 0.05). There were also significant correlations of Vc/Va both before and after bronchodilator with FEV1, MMFR25/75 and before bronchodilator with RV/TLC in the SCD children (Table 5) but no significant correlations of Vc/Va with any of the lung function test results in the controls (Table 6).

DISCUSSION

We have demonstrated that SCD children compared to ethnic origin matched controls had higher respiratory system resistance and pulmonary capillary blood volume both before and after bronchodilator. Respiratory system resistance and pulmonary capillary blood volume results were significantly correlated in the SCD children, but not in the controls, suggesting the raised respiratory system resistance in the SCD children was, at least partly, explained by their increased pulmonary capillary blood volume. Furthermore, the SCD children who had elevated RSHs Speak results compared to those who did not had significantly higher Vc/Va results. These latter findings, however, should be interpreted with the caveat that, in the absence of ethnic specific RSHs reference ranges, we used the data from our controls to define an abnormal RSHs result. In the 50 children overall, pulmonary capillary blood volume correlated with the impulse oscillometry results and other measures of airway obstruction. In addition, we demonstrated a significant negative correlation between the pulmonary capillary blood volume and vital capacity. The raised pulmonary capillary blood volume seen in the SCD children is a response to their chronic anemia resulting in a raised cardiac output and increased pulmonary blood volume and vascular recruitment. The increase in pulmonary blood volume resulting from the increase in cardiac output due to their chronic anemia may explain the relatively normal DLCO results we report despite lower lung volumes. Our results are consistent with those results from adults with SCD highlighting they had a raised pulmonary capillary blood volume. The significant correlation of the respiratory

![Image](image.png)

**Figure 1. RSHs Speak and pulmonary capillary blood volume (Vc/Va). Individual data points are shown. •, SCD children; ○, controls.**

TABLE 5—Correlations Between Vc/Va and Lung Function Results in the SCD Children

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-bronchodilator</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RS</td>
<td>0.328</td>
<td>0.0069</td>
</tr>
<tr>
<td>FEV1</td>
<td>−0.425</td>
<td>0.0062</td>
</tr>
<tr>
<td>VC</td>
<td>−0.285</td>
<td>0.1786</td>
</tr>
<tr>
<td>FEV1/VC</td>
<td>−0.300</td>
<td>0.0864</td>
</tr>
<tr>
<td>MMFR25/75</td>
<td>−0.445</td>
<td>0.0048</td>
</tr>
<tr>
<td>RV</td>
<td>0.243</td>
<td>0.2449</td>
</tr>
<tr>
<td>RV/TLC</td>
<td>0.670</td>
<td>0.0020</td>
</tr>
<tr>
<td>TLC</td>
<td>−0.148</td>
<td>0.4910</td>
</tr>
<tr>
<td>Post-bronchodilator</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RS</td>
<td>0.482</td>
<td>0.0148</td>
</tr>
<tr>
<td>FEV1</td>
<td>−0.450</td>
<td>0.0075</td>
</tr>
<tr>
<td>VC</td>
<td>−0.315</td>
<td>0.1332</td>
</tr>
<tr>
<td>FEV1/VC</td>
<td>−0.239</td>
<td>0.2602</td>
</tr>
<tr>
<td>MMFR25/75</td>
<td>−0.407</td>
<td>0.0042</td>
</tr>
</tbody>
</table>

RS, resistance from impulse oscillometry at 5 Hz; FEV1, forced expiratory volume in 1 sec; VC, vital capacity; FEV1/VC, forced expiratory volume in 1 sec: vital capacity; MMFR25/75, mean maximum expiratory flow; RV, residual volume; RV/TLC, residual volume/total lung capacity.

All results were expressed as the percentage predicted for height.  

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system resistance results and the hemoglobin levels emphasize the relationship between the levels of anemia, pulmonary capillary blood volume, and peripheral airway obstruction. The effect of transfusions on lung function to further tease out the etiology of airways obstruction in SCD children merits testing.

The SCD children had a significantly higher mean RS% predicted than the controls indicating they had a higher respiratory system resistance, a finding consistent with studies showing obstructive abnormalities in SCD children.27 Other assessments of lung function demonstrated that the SCD children compared to the controls had significantly greater obstructive and restrictive abnormalities. The controls had similar changes in respiratory system resistance and other measures of airways obstruction post-bronchodilator to the SCD children suggesting that their greater airway obstruction abnormalities may be due to their increased pulmonary capillary blood volume.

Strengths of this study are that comparison was made of SCD children to ethnic matched controls who were of similar age. It is possible if we had used a beta-2 agonist rather than ipratropium bromide we may have had different results. An anticholinergic bronchodilator, ipratropium bromide, however, was used as it has small and clinically unimportant hemodynamic effects15 and thus would be unlikely to influence pulmonary capillary blood volume. Indeed, no change in pulmonary capillary blood volume was seen after bronchodilator (ipratropium bromide) administration. A degree of bronchial tone occurs even in healthy individuals, thus we were not surprised to see a decrease in RS, but not in FEV1, after bronchodilator administration; an IOS is a more sensitive test than spirometry.16 Although, overall, the changes in lung function were statistically significant, the magnitude of changes was small and unlikely to be of clinical significance. Impulse oscillometry was used to assess respiratory system resistance, as this technique has been found to be more sensitive in assessing peripheral airway function than spirometry.17 The lung function results were related to reference ranges derived from caucasian subjects,18 but the two groups were matched for ethnic origin and the reference ranges were used in both groups, thus comparisons between them were valid. The impulse oscillometry results showing a correlation with pulmonary capillary blood volume were supported by the spirometric data demonstrating other measures of airways obstruction correlated with pulmonary capillary blood volume. We did not take blood samples to assess hemoglobin levels in the controls, but the levels determined using the Hb pulse oximetry were within the normal range, as would be expected in healthy controls. In addition, non-invasive measurement of hemoglobin concentration has been validated in healthy adult volunteers undergoing hemodialysis19 and in adult surgical and critical care patients20-21.

In conclusion, our results suggest that the raised pulmonary capillary blood volume seen in SCD children may, at least partially, explain their increased airways resistance. Our findings may pertain mainly to younger children in whom obstructive patterns of lung function are more prevalent. Whether these effects persist in an older cohort with restrictive lung disease merits study. A clinical implication of these results is that SCD children with airways obstruction may only have limited benefit from bronchodilators. Strategies to reduce anemia and increased pulmonary capillary blood volume, such as hydroxyurea or blood transfusion, may be beneficial in those who remain symptomatic despite optimization of bronchodilator therapy and merits testing.

ACKNOWLEDGMENTS

This research was supported by the National Institute for Health Research (NIHR) Biomedical Research Centre at Guy’s and St Thomas’ NHS Foundation Trust and King’s College London. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health, A.G. is an NIHR Senior Investigator and an MRC and Asthma UK Centre in Allergic Mechanisms of Asthma Investigator, supported by MRC Centre Grant G1000758. Funding for consumables was provided by the Isaac Schapera Research Trust and the equipment to assess peripheral airways resistance was provided by a grant from the King’s College Hospital NHS Foundation Trust Charity.
REFERENCES


11. Appendix B: SPSS syntax for mixed-effect models

DATASET ACTIVATE DataSet5.
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   /CRITERIA=CIN(95) MXITER(100) MXSTEP(10) SCORING(1) SINGULAR(0.0000000000001) HCONVERGE(0).
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   /SAVE=PRED RESID.
   /EMMEANS=TABLES(SCStatus) COMPARE ADJ(LSD).

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   /CRITERIA=CIN(95) MXITER(100) MXSTEP(10) SCORING(1) SINGULAR(0.0000000000001) HCONVERGE(0).
   /PRINT=TESTCOV SOLUTION.
   /SAVE=PRED RESID.
   /EMMEANS=TABLES(SCStatus2) COMPARE ADJ(LSD).

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   /PRINT=TESTCOV SOLUTION.
   /SAVE=PRED RESID.
   /EMMEANS=TABLES(SCStatus3) COMPARE ADJ(LSD).

MIXED TLC BY SCStatus WITH AgeGroupmeanCentered
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   /PRINT=TESTCOV SOLUTION.
   /SAVE=PRED RESID.
   /EMMEANS=TABLES(SCStatus) COMPARE ADJ(LSD).

MIXED TLC BY SCStatus2 WITH AgeGroupmeanCentered
/CRITERIA=CIN(95) MXITER(100) MXSTEP(10) SCORING(1) SINGULAR(0.000000000001) HCONV E(0).
   /ABSOLUTE LCONVERGE(0. ABSOLUTE) PCONVERGE(0.000001 , ABSOLUTE)
   /FIXED=Age Group meanCentered SCDataType3 SCDataType3*Age Group meanCentered | SSTYPE(3)
   /METHOD=REML
   /PRINT=SOLUTION TESTCOV
   /RANDOM=INTERCEPT Age Group meanCentered | SUBJECT(id) COVTYPE(AR1)
   /REPEATED=Age Group meanCentered | SUBJECT(id) COVTYPE(AR1)
   /SAVE=FIXPRED PRED RESID.

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   /ABSOLUTE LCONVERGE(0. ABSOLUTE) PCONVERGE(0.000001 , ABSOLUTE)
   /FIXED=Age Group meanCentered SCDataType3 SCDataType3*Age Group meanCentered | SSTYPE(3)
   /METHOD=REML
   /PRINT=SOLUTION TESTCOV
   /RANDOM=INTERCEPT Age Group meanCentered | SUBJECT(id) COVTYPE(AR1)
   /REPEATED=Age Group meanCentered | SUBJECT(id) COVTYPE(AR1)
   /SAVE=FIXPRED PRED RESID
   /EMMEANS=TABLES(SCDataType3) COMPARE ADJ(LSD).

DATASET ACTIVATE DataSet7.
MIXED TLC BY SCDataType WITH Time
/CRITERIA=CIN(95) MXITER(100) MXSTEP(10) SCORING(1) SINGULAR(0.000000000001) HCONV E(0).
   /ABSOLUTE LCONVERGE(0. ABSOLUTE) PCONVERGE(0.000001 , ABSOLUTE)
   /FIXED=Time SCDataType SCDataType*Time | SSTYPE(3)
   /METHOD=REML
   /PRINT=SOLUTION TESTCOV
   /RANDOM=INTERCEPT Time | SUBJECT(id) COVTYPE(AR1)
   /REPEATED=Time | SUBJECT(id) COVTYPE(AR1)
   /SAVE=FIXPRED PRED RESID
   /EMMEANS=TABLES(SCDataType) COMPARE ADJ(LSD).
DATASET ACTIVATE DataSet1.
MIXED TLC BY SCD WITH AgeRescaled
/Criteria=Line(5) MXITER(100) MXSTEP(10) SCORING(1) SINGULAR(0,000000000001) HCONVERGE(0).
/ABSOLUTE LCONVERGE(0, ABSOLUTE) PCONVERGE(0,000001, ABSOLUTE)
/FIXED=AgeRescaled SCD SCD*AgeRescaled | SSTYPE(3)
/METHOD=REML
/PRINT=SOLUTION TESTCOV
/RANDOM=INTERCEPT AgeRescaled | SUBJECT(id) COVTYPE(ID)
/REPEATED=AgeRescaled | SUBJECT(id) COVTYPE(ID)
/SAVE=FIXPRED PRED RESID
/EMMEANS=TABLES(SCD) WITH(AgeRescaled=6) COMPARE ADJ(LSD).
DATASET ACTIVATE DataSet1.
MIXED R/vpp BY SCDcontrolReference ACSnolACSreference ObstructiveNoObstructionReference_Null
PtorACSnolPtorACSreference WITH AgeCentered_9 3
/Criteria=CLIN(95) MXITER(1000) MXSTEP(10) PCONVERGE(0.000001) SCORING(1) SINGULAR(0.000000000001) HCOV
GE(0).
/FIXED=AgeCentered_9 3 ACSnolACSreference(SCDcontrolReference)
ObstructiveNoObstructionReference_Null(SCDcontrolReference)
PtorACSnolPtorACSreference*AgeCentered_9 3(SCDcontrolReference)
ACSnolACSreference*AgeCentered_9 3(SCDcontrolReference)
ObstructiveNoObstructionReference_Null*AgeCentered_9 3(SCDcontrolReference)
PtorACSnolPtorACSreference*AgeCentered_9 3(SCDcontrolReference) | SSTYPE(3)
/METHOD=REML.
/PRINT=SOLUTION TESTCOV
/RANDOM=INTERCEPT AgeCentered_9 3 | SUBJECT(id) COVTYPE(1)
/EMMEANS=TABLES (OVERALL).

MIXED R/vpp BY SCDcontrolReference ACSnolACSreference ObstructiveNoObstructionReference_Null
PtorACSnolPtorACSreference WITH AgeCentered_9 3
/Criteria=CLIN(95) MXITER(1000) MXSTEP(10) SCORING(1) SINGULAR(0.000000000001) HCOV
GE(0).
/FIXED=AgeCentered_9 3 ACSnolACSreference(SCDcontrolReference)
ObstructiveNoObstructionReference_Null(SCDcontrolReference)
PtorACSnolPtorACSreference*AgeCentered_9 3(SCDcontrolReference)
ACSnolACSreference*AgeCentered_9 3(SCDcontrolReference)
ObstructiveNoObstructionReference_Null*AgeCentered_9 3(SCDcontrolReference)
PtorACSnolPtorACSreference*AgeCentered_9 3(SCDcontrolReference) | SSTYPE(3)
/METHOD=REML.
/PRINT=SOLUTION TESTCOV
/RANDOM=INTERCEPT AgeCentered_9 3 | SUBJECT(id) COVTYPE(1)
/EMMEANS=TABLES (OVERALL).

MIXED R/vpp BY SCDcontrolReference ACSnolACSreference ObstructiveNoObstructionReference_Null
PtorACSnolPtorACSreference WITH AgeCentered_9 3
/Criteria=CLIN(95) MXITER(1000) MXSTEP(10) SCORING(1) SINGULAR(0.000000000001) HCOV
GE(0).
/FIXED=AgeCentered_9 3 ACSnolACSreference(SCDcontrolReference)
ObstructiveNoObstructionReference_Null(SCDcontrolReference)
PtorACSnolPtorACSreference*AgeCentered_9 3(SCDcontrolReference)
ACSnolACSreference*AgeCentered_9 3(SCDcontrolReference)
ObstructiveNoObstructionReference_Null*AgeCentered_9 3(SCDcontrolReference)
PtorACSnolPtorACSreference*AgeCentered_9 3(SCDcontrolReference) | SSTYPE(3)
/METHOD=REML.
/PRINT=SOLUTION TESTCOV