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Abnormal Pulmonary Function in Adults with Sickle Cell Anemia

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Abstract:

Rationale: Pulmonary complications of sickle cell anemia (Hb-SS) commonly cause morbidity, yet few large studies of pulmonary function tests (PFTs) in this population have been reported.

Objectives: PFTs [spirometry, lung volumes and diffusion capacity for carbon monoxide (D_LCO)] from 310 Hb-SS adults were analyzed to determine the pattern of pulmonary dysfunction and their association with other systemic complications of sickle cell disease.

Methods: Raw PFT data were compared with predicted values. Each subject was sub-classified into 1 of 5 groups: obstructive physiology; restrictive physiology; mixed obstructive/restrictive physiology; isolated low D_LCO; or normal. The association of laboratory data between patients with a decreased D_LCO or restrictive physiology compared with normals was assessed by multivariate linear regression.

Measurements and Main Results: Normal PFTs were present in only 31 of 310 (10%) patients. Overall, adult Hb-SS was characterized by decreased total lung capacities (70.2 $\pm$ 14.7% predicted) and D_LCO (64.5 $\pm$ 19.9 %). The most common PFT patterns were restrictive physiology (74%), and an isolated low D_LCO (13%). Decreased D_LCO was associated with thrombocytosis (p=0.05), and with hepatic [elevated ALT (p=0.07)] and a trend towards renal dysfunction [elevated BUN and creatinine (p=0.05, 0.07)].

Conclusions: Pulmonary function is abnormal in 90% of adult Hb-SS patients. Common abnormalities include restrictive physiology and a decreased D_LCO. A decreased D_LCO may indicate more severe sickle vasculopathy characterized by impaired hepatic and renal function.

Abstract Word Count: 218

Key words: restrictive disease, dyspnea, airway obstruction

Introduction:

Sickle cell anemia (Hb-SS) results from homozygosity for a point mutation in the β-globin gene (*HBB*; glu6val) causing the resultant sickle hemoglobin (Hb-S) to be less soluble when deoxygenated than normal hemoglobin (1). Even with improved treatment, including the early use of prophylactic antibiotic regimens, judicious transfusions and the administration of hydroxyurea in selected patients, mortality remains high for this population. The median age at death is 42 years for males and 48 years for females with Hb-SS. Pulmonary complications, including acute chest syndrome (ACS), pulmonary hypertension (PH), and pulmonary fibrosis, account for 20-30% of deaths in the Hb-SS population and are often under-recognized by the health care community (2,3).

Dyspnea is a frequent complaint amongst patients with sickle cell disease, the etiology of which is unclear and likely multi-factorial (4,5). Studies of lung function to date in this population have been of modest size, often involving less than 50 patients (4,6-11) and largely inconclusive. Their results have yielded a spectrum of abnormalities including restrictive lung disease, abnormal diffusion capacity for carbon monoxide (D_LCO), obstructive disease and hypoxemia (4,6,7,9,12). No definitive profile for pulmonary function in sickle cell disease has emerged. As a result, clinicians find pulmonary function tests (PFTs) difficult to interpret in this population and their clinical utility for directing further investigation and therapy has not been well evaluated.

Of growing concern is the link between obstructive lung disease and ACS, particularly in children (7,13,14). The few published studies suggest that obstructive lung disease, in some cases, plays a role in the pathogenesis of ACS (7,13,15). Moreover, obstructive lung disease could be a long-term sequelae of recurrent episodes of ACS (6,9). Larger scale studies are necessary to elucidate more clearly the interaction between lung function and ACS. Additionally, certain findings on PFTs might be a marker of other complications of sickle vasculopathy. For example, an isolated decreased D_LCO is a well-established finding associated with PH (16,17). However, its role as a marker or predictor of PH in the Hb-SS population is unknown. The purpose of this study is to evaluate the relation of the demographic, clinical and laboratory characteristics to lung function in the SCD population. PFTs were done on subjects recruited as part of the Cooperative Study of Sickle Cell Disease (CSSCD), a cross-sectional epidemiological study. Some of the results of these studies have been previously presented in the form of an abstract at the 47th annual meeting of the American Society of Hematology (18).

Methods:

Patient Database: PFTs were collected as part of the CSSCD which enrolled and followed over 4,000 patients with SCD evaluated at one of 23 participating clinical centers across the United States between 1978 and 1998 (19,20). One of the primary goals of the CSSCD was to collect data on the clinical course of SCD from birth to death. From the original study population, only the 2061 (51%) subjects with sickle cell anemia were included in this study. The study population was further limited by inclusion of only those subjects who were African-American and over the age of 18 years. A history of acute chest syndrome (ACS) was assessed as part of the original data collection. ACS was defined as a) a new infiltrate on chest radiograph or b) pleuritic chest pain and/or dyspnea in addition to an abnormal ventilation-perfusion scan. As part of the standard protocol established by the CSSCD, each subject underwent pulmonary function testing three times to ensure reproducibility. If the results from the three studies differed by more than 2%, the study was considered to be of poor quality. Prior to entering into the database, all PFTs were reviewed for quality by pulmonologists at either Harlem Hospital in New York, NY or Howard University Medical Center in Washington, DC as part of the standard protocol. Upon our review of the data, we considered data insufficient if the evaluation was considered poor quality or if more than 25% of the data for a subject was missing, suggesting an inability to perform the tests correctly; these cases were subsequently removed from our database. Review of these data was approved by the Institutional Review Board of Boston University/Boston Medical Center.

Pulmonary Function Testing: Pulmonary function tests were obtained from patients clinically in the steady-state (at least 4 weeks after a vasoocclusive crisis). Each subject underwent spirometry [forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), and FEV_1/FVC], lung volumes [total lung capacity (TLC) and residual volume (RV)] and D_LCO according to the standard protocols employed by each of the 23 centers of the CSSCD. D_LCO values were corrected for the serum hemoglobin concentration obtained at the time of PFT testing utilizing previously published formulas (21). They were not corrected for alveolar volumes as these data were not available. Predicted values for FEV_1, FVC, FEV_1/FVC, TLC, RV and D_LCO were calculated using algorithms that accounted for gender, age, and height in the African American population (STATA, version 9); data are presented as percent predicted (22-24).

Classification of Pulmonary Function: The pulmonary function of each subject was classified into one of five categories based upon ATS criteria (25). The five categories were: 1) Normal; 2) Obstructive; 3) Restrictive; 4) Mixed obstructive and restrictive; 5) Isolated low D_LCO based upon the following criteria:

1) Normal: FEV_1, FVC, TLC, RV, D_LCO within the normal range ($\geq$80% predicted) with FEV_1/FVC $\geq$ 70%.
2) Obstructive: A FEV_1/FVC ratio of < 70%, associated with decreased FEV_1 and FVC (<80% predicted). TLC and RV were either normal or elevated ($\geq$120% predicted). D_LCO was normal ($\geq$ 80% predicted).
3) Restrictive: a) FEV_1, FVC and TLC were decreased ($\leq$80% predicted) with normal FEV_1/FVC ratio ($\geq$70%) and a decrease in D_LCO ($\leq$80% predicted) or b)

TLC and RV were decreased (≤80% predicted) with normal D_LCO, FEV_1, FVC and FEV_1/FVC suggestive of low lung volumes (LLV) or c) Reduced TLC and D_LCO.

4) Mixed obstructive and restrictive: FEV_1/FVC ratio was reduced suggestive of obstructive disease. TLC and RV were reduced suggestive of restrictive disease. D_LCO was normal.
5) Isolated low D_LCO: D_LCO was decreased with normal FEV_1, FVC, FEV_1/FVC, TLC and RV.

Statistical Analysis: Socio-demographic and laboratory measures are presented as means ± standard deviations (SD). PFT data are presented as means ± SD, and medians according to their percent predicted. The association of selected laboratory measures was determined by comparing those patients with isolated low D_LCO and restrictive disease to those with normal D_LCO measures and normal PFTs, respectively. Analysis of variance (ANOVA) was used to calculate both the unadjusted and age-adjusted means for each laboratory measure and these means were compared using an *F* test (SAS software version 8.2).

Results:

Patient Characteristics (Table 1): 310 adult subjects were eligible for this study after subjects who were not African American, homozygous for HbS and those without complete PFTs were excluded. The average age of the subjects was 30.7 ±10.3 years (range 20-67 years) and 41% were males. Subjects had a normal body habitus as reflected by a body mass index of 21.3 ± 4.2. ACS was common in this population, with 37% of subjects reporting a history of ACS prior to enrollment in the study and an additional 38% reported an incident episode during the follow-up period.

Laboratory Values (Table 1): The hematological profile of the subjects was reflective of adults with Hb-SS. Anemia (Hb = 8.25 ± 1.44 g/dl), leukocytosis (WBC = 11.75 ± 2.64 cells/L/mm^3) and thrombocytosis (platelet count = 410.96 ± 113.46 x 10^5/ml) were present. There was evidence of ongoing hemolysis characterized by an elevation in lactate dehydrogenase (LDH) (497.39 ± 166.67 U/L) (normal 100-250 U/L), aspartate aminotransferase (AST) (47.24 ± 23.93 U/L) (normal 5-40 U/L) and bilirubin (3.62 ± 2.13 mg/dl) (normal 0.3-1.2 mg/dl) concentrations (26). There was a mild elevation of the hepatic enzyme alanine aminotransferase (ALT) (44.90 ± 52.31 U/L). Renal function as assessed by serum creatinine concentrations were within normal laboratory ranges in this population; however, creatinine is now known to be an insensitive measure of glomerular filtration rate in Hb-SS (27).

Pulmonary Function in Hb-SS Patients (Table 2): Abnormal pulmonary function was observed in 90% (279/310) of the subjects. Overall, the population was characterized

by restrictive physiology with decreased TLC (70.2 ± 14.69% predicted) and D_LCO (56.57 ± 20.11%). Although the spirometry was technically within the normal range, the means for FEV_1 (83.03 ± 16.06 % predicted) and FVC (84.37 ± 16.01% predicted) were considered to be low-normal with an FEV_1/FVC of 98.36 ± 9.15%. The decreased D_LCO persisted after adjustment for the patients' hemoglobin concentration (64.54 ± 19.93% predicted). Sub-categorization of the patients revealed that the most common PFT abnormalities observed were restrictive disease (74%) and an isolated low D_LCO (13%). Obstructive disease, either alone or in conjunction with restrictive disease, was relatively uncommon, occurring in 3% of the patients.

Effect of ACS on Lung Function (Table 3): 221 of the 310 subjects (71.3%) had at least one episode of ACS prior to obtaining pulmonary function studies. Overall, this group was characterized by a trend towards lower TLC (69.17 vs. 72.83%, p=0.06) and adjusted D_LCO (63.32 vs. 67.81, p=0.10) compared to those without a history of ACS. The patterns of pulmonary function abnormalities observed in those with a history of ACS were similar to those without a history of ACS with restrictive disease and an abnormal diffusion capacity predominating.

Decreased D_LCO is Associated with Increasing Age (Figure 1): As our population did not have a predominance of obstructive disease as observed in other studies of the pediatric population (7,13-15), we evaluated the effects of age on TLC and D_LCO. Interestingly, a negative linear correlation existed between D_LCO and age suggesting that in Hb-SS adults, disruption of alveolar-capillary gas exchange may be a potential

mechanism for hypoxemia in older Hb-SS adults. There was no association between TLC and age in this population (data not shown).

Association of Restrictive Lung Disease with Severity of Hb-SS (Table 4): The multivariate regression analysis adjusting for age resulted in a trend towards association between the presence of restrictive lung disease and a more severe Hb-SS phenotype. This phenotype is characterized by more severe anemia (Hb concentrations 8.3 g/dl vs. 8.7 g/dl, p=0.08, HCT 24.7% vs. 26.2%, p=0.05) and leukocytosis (WBC 11.93 x 10^3 vs. 10.95 x 10^3, p=0.06), compared with Hb-SS patients with normal PFTs. Additional linear regression analysis revealed a negative correlation between TLC and WBC count (r=-0.15, p=0.01) (data not shown).

Association of a Decreased D_LCO with Systemic Disease (Table 4 and Figure 2): After adjustment for age, the presence of a low D_LCO was associated with an increase in platelet counts (441,000 vs. 391,900, p=0.05) and blood-urea-nitrogen (BUN) concentrations (10.9 mg/dl vs. 8.3 mg/dl, p=0.05), compared with those with normal D_LCO or normal PFTs. There was a trend towards increased ALT (59.2 U/L vs. 31.1 U/L, p=0.08) and creatinine concentrations (1.11 mg/dl vs. 0.8 mg/dl, p=0.07) in the patients with a decreased D_LCO compared with those with normal D_LCO. Additionally, linear regression revealed a negative correlation between DLCO and both BUN (r=-0.22, p=0.0002) and creatinine (r=-0.22, p=0.0003) concentrations, suggesting that worsening diffusion capacity may be a marker for systemic vasculopathy. Interestingly, there was no correlation between D_LCO and the severity of anemia. The uncorrected

D_LCO had a positive linear association with the Hb concentration (Figure 2), as would be expected in a non-SCD population, suggesting that correcting the D_LCO for the level of anemia in our study did not falsely modify the results.

Discussion:

Pulmonary complications of adult Hb-SS are responsible for 20-30% of the mortality observed in this population (2,3,8). Although PH occurs in about one-third of patients with Hb-SS (28), it is not the only etiology of dyspnea observed in this population. We and others have found that at least one-half of adults with sickle cell disease are at least mildly dyspneic and that dyspnea, in some cases, is not associated with echocardiographic evidence of PH (4, unpublished observations Klings et al.). Prior studies have suggested that abnormal pulmonary function tests are the first objective sign of chronic sickle cell lung disease and that they could be helpful in patient management (8). Previously, multiple small studies have demonstrated a spectrum of PFT abnormalities in adult sickle cell disease including restrictive physiology, a decreased D_LCO, hypoxemia and obstructive disease (4,5,8,10,12,26). Our analysis is the first large-scale multi-center effort to report PFTs in adults with Hb-SS. Pulmonary function was abnormal in 90% of our cases and PFTs may allow clinicians to objectively assess dyspnea in this population.

The most common PFT abnormality observed was restrictive disease. According to the ATS guidelines, the best diagnostic test for the presence of restrictive disease is a TLC < 80% predicted (25). When we examined the group of patients with restrictive disease, an interesting pattern emerged. Of the 230 subjects with restrictive disease, only 111 (48.3%) had the full constellation of low TLC, abnormal spirometry (reduced FEV_1 and FVC, with a $FEV_1/FVC \geq 70\%$) and reduced D_LCO. The remaining subjects were characterized by low lung volumes with normal spirometry. The categorization of these

subjects together reflects the fact that a low TLC alone is enough to make a diagnosis of restriction (25). Our population demonstrates a divergence of spirometry and lung volumes contradicting other studies in the literature. Previously, in a large-scale study of a healthy Caucasian population, only 2.4% of subjects with a normal FVC had restrictive disease and it was recommended that spirometry alone was sufficient to exclude the presence of restriction unless a high degree of clinical suspicion was present (29). In Hb-SS, it appears clear that spirometry alone is not sufficient to predict the presence of restrictive lung disease.

One explanation for this may be racial differences inherent to the sickle cell population. Race is an important determinant of lung function. Previous studies have demonstrated that, on average, equations for predicted values of PFT data based upon data generated in Caucasians over-estimate the TLC, FEV_1, and FVC by 12% and the RV and functional residual capacity (FRC) by 7% (25). Although all of our data were corrected for race prior to analysis, there may still be a differential pattern present. Another possible explanation may stem from extra-pulmonary causes of restrictive physiology. In the Hb-SS population, one extra-pulmonary mechanism of restriction would be ineffective inspiration due to chest wall pain related to peripheral vasoocclusion, prior rib infarctions or vertebral disease (30). Although the PFTs obtained in this study were done while the subjects were clinically at their baseline, even when clinically well, Hb-SS patients may have sub-acute vasoocclusion (31). The amount of chest wall discomfort experienced by the subjects at the time of their testing is unknown but could have contributed to the low lung volumes observed. Additionally,

there may be differences in chest wall and vertebral structure among adults with Hb-SS contributing to the restrictive physiology. This could be due to repeated bony infarctions during growth and development and the spinal osteoporosis and osteomalacia typical of Hb-SS (32-34). Non-SCD specific etiologies of extra-pulmonary restriction such as obesity may be playing a role in the restrictive physiology observed in a subset of the subjects observed. The presence of restrictive physiology was associated with a trend towards more severe clinical disease, as exemplified by lower total hemoglobin concentrations and hematocrits, leukocytosis and renal dysfunction (elevated BUN and creatinine) (Table 4). The association of leukocytosis with a decrease in the TLC supports the notion that restrictive lung disease occurs as a result of repeated episodes of ACS and may be a risk factor for mortality (2). The occurrence of more frequent episodes of ACS may be a risk factor for the development of pulmonary fibrosis, a common etiology of restrictive physiology on PFTs. Additionally, this suggests that the patients with restrictive lung disease have a greater propensity towards vasoocclusion placing them at increased risk for the development of bony infarcts. Interestingly, we noted an association between chronic organ dysfunction of the lungs and the kidneys in our population, confirming findings of prior studies and suggesting an etiologic link between these two entities (35).

An equally interesting finding is the presence of a decreased D_LCO in Hb-SS adults. In 13% of subjects, this was the only PFT abnormality observed. An isolated decreased D_LCO can be a marker for early interstitial lung disease (36) or more commonly, is associated with the presence of PH. In patients with systemic sclerosis, a $D_LCO \leq 55\%$

predicted may be observed in patients up to 5 years prior to the diagnosis of PH and is thought to be an early screening tool in this population (17,37). Moreover, in patients with idiopathic pulmonary arterial hypertension (IPAH), a low D_LCO is not only a marker of PH, but is also an independent predictor of mortality (16). Unfortunately, as the CSSCD was designed prior to the determination of the importance of PH in the natural history of SCD, echocardiographic data is not available on the majority of the study subjects. Because of this, a definitive conclusion about the relationship between a reduced D_LCO and the presence of PH cannot be made. We recognize that this is a shortcoming of the current study and recommend a longitudinal study evaluating pulmonary function tests and echocardiograms in Hb-SS adults to clarify this issue.

Age-adjusted multivariate analysis revealed a link between the presence of a low D_LCO with markers of hepatic and renal dysfunction and with thrombocytosis. Prior studies of PH in Hb-SS have revealed an association of PH with a history of renal dysfunction and an elevation in ALT (28,38). Additionally, platelets appear to have an important role pathogenically in the development of PH both as mediators of serotonin metabolism and in their role in thrombosis. These observations support the notion that a decreased D_LCO may be associated with PH in the SCD population. Unfortunately, the lack of correlative echocardiographic data limits our ability to draw definitive conclusions about the link between a decreased D_LCO and PH in this population.

Limitations of the current analysis stem from the study design. As this was a cross-sectional study of pulmonary function data, it is impossible to assess the longitudinal

effects of Hb-SS disease on pulmonary function. The lack of a non-SCD control group also limits the interpretability of this study somewhat. It would be interesting to determine if the apparent obstructive disease observed in the pediatric Hb-SS population undergoes a transition to a more restrictive pattern in adulthood. Additionally, testing for airway hyperreactivity, such as response to inhaled bronchodilators or methacholine challenge testing was not done. Its possible that subjects with co-existent asthma may not have been detected in the current study as airflow typically normalizes in these patients between acute exacerbations. A large-scale prospective study of patients with Hb-SS would be helpful in determining the association of asthma with Hb-SS, the role of asthma in the development of sickle cell lung disease, and the usefulness of the presence of a decreased D_LCO as a marker for PH. Additionally, further work needs to be performed to more clearly delineate the etiologies of restrictive disease in Hb-SS, the potential role that parenchymal lung disease plays in the pathogenesis of hypoxemia in this population, and the extent and severity of PFT abnormalities in Hb-SC disease (compound heterozygosity for HbS and HbC [*HBB* glu6lys]), another common genotype of sickle cell disease.

In conclusion, pulmonary function is abnormal in 90% of adults with Hb-SS. It is likely that abnormal pulmonary function reflects intrinsic lung disease in these patients and that the mechanisms of dyspnea are more complex in this population than originally appreciated. Greater understanding of the diagnostic utility of pulmonary function testing in this population is paramount as it could lead to a more comprehensive appraisal of the mechanisms responsible for dyspnea and hypoxemia.

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References:

1. Eaton WA, H. J. 90 A.D. Sickle cell hemoglobin polymerization. Adv Protein Chem,63 ed. 280.
2. Platt, O. S., D. J. Brambilla, W. F. Rosse, P. F. Milner, O. Castro, M. H. Steinberg, and P. P. Klug. 1994. Mortality in sickle cell disease. Life expectancy and risk factors for early death. *N.Engl.J.Med.* 330:1639-1644.
3. Steinberg, M. H., F. Barton, O. Castro, C. H. Pegelow, S. K. Ballas, A. Kutlar, E. Orringer, R. Bellevue, N. Olivieri, J. Eckman, M. Varma, G. Ramirez, B. Adler, W. Smith, T. Carlos, K. Ataga, L. DeCastro, C. Bigelow, Y. Saunthararajah, M. Telfer, E. Vichinsky, S. Claster, S. Shurin, K. Bridges, M. Waclawiw, D. Bonds, and M. Terrin. 2003. Effect of hydroxyurea on mortality and morbidity in adult sickle cell anemia: risks and benefits up to 9 years of treatment. *JAMA* 289:1645-1651.
4. Delclaux C, Zerah-Lancner F, Bachir D, Habibi A, Monin JL, Godeau B and Galacteros F. 2005. Factors associated with dyspnea in adult patients with sickle cell disease. *Chest* 128:3336-3344.
5. Miller GJ, Serjeant GR. 1971. An assessment of lung volumes and gas transfer in sickle-cell anaemia. *Thorax*; 26:309-315.
6. Santoli, F., F. Zerah, N. Vasile, D. Bachir, F. Galacteros, and G. Atlan. 1998. Pulmonary function in sickle cell disease with or without acute chest syndrome. *Eur.Respir.J.* 12:1124-1129.
7. Leong, M. A., C. Dampier, L. Varlotta, and J. L. Allen. 1997. Airway hyperreactivity in children with sickle cell disease. *J.Pediatr.* 131:278-283.

8. Powars, D., J. A. Weidman, T. Odom-Maryon, J. C. Niland, and C. Johnson. 1988. Sickle cell chronic lung disease: prior morbidity and the risk of pulmonary failure. *Medicine (Baltimore)* 67:66-76.
9. Girgis RE, Qureshi MA, Abrams J, Swerdlow P. 2003. Decreased exhaled nitric oxide in sickle cell disease: relationship with chronic lung involvement. *Am J Hematol* 72: 177-184.
10. Jaja SI, Opesanwo O, Mojiminivi FB, Kehinde MO. Lung function, haemoglobin and irreversibly sickled cells in sickle cell patients. 2000. *West Afr J Med* 19:225-229.
11. Femi-Pearse D, Gazioglu KM, Yu PN. 1970. Pulmonary function studies in sickle cell disease. *J Appl Physiol* 28:574-577.
12. Machado, R. F. and M. T. Gladwin. 2005. Chronic sickle cell lung disease: new insights into the diagnosis, pathogenesis and treatment of pulmonary hypertension. *Br.J.Haematol.* 129:449-464.
13. Knight-Madden, J. M., T. S. Forrester, N. A. Lewis, and A. Greenough. 2005. Asthma in children with sickle cell disease and its association with acute chest syndrome. *Thorax* 60:206-210.
14. Koumbourlis, A. C., H. J. Zar, A. Hurlet-Jensen, and M. R. Goldberg. 2001. Prevalence and reversibility of lower airway obstruction in children with sickle cell disease. *J.Pediatr.* 138:188-192.
15. Sylvester, K. P., R. A. Patey, P. Milligan, M. Dick, G. F. Rafferty, D. Rees, S. L. Thein, and A. Greenough. 2004. Pulmonary function abnormalities in children with sickle cell disease. *Thorax* 59:67-70.

16. D'Alonzo, G. E., R. J. Barst, S. M. Ayres, E. H. Bergofsky, B. H. Brundage, K. M. Detre, A. P. Fishman, R. M. Goldring, B. M. Groves, J. T. Kernis, and . 1991. Survival in patients with primary pulmonary hypertension. Results from a national prospective registry. *Ann.Intern.Med.* 115:343-349.

17. Mukerjee, D., D. St George, C. Knight, J. Davar, A. U. Wells, R. M. Du Bois, C. M. Black, and J. G. Coghlan. 2004. Echocardiography and pulmonary function as screening tests for pulmonary arterial hypertension in systemic sclerosis. *Rheumatology.(Oxford)* 43:461-466.

18. Klings ES, Wyszynski DF, Nolan VG, Steinberg MH. 2005. Abnormal pulmonary function in adults with sickle cell disease: association of decreased D_LCO with systemic disease (abstract). *Blood* (ASH annual meeting abstracts) 106:316.

19. Gaston, M., J. Smith, D. Gallagher, Z. Flournoy-Gill, S. West, R. Bellevue, M. Farber, R. Grover, M. Koshy, A. K. Ritchey, et al. 1987. Recruitment in the Cooperative Study of Sickle Cell Disease (CSSCD). *Control Clin.Trials* 8:131S-140S.

20. Gaston, M. and W. F. Rosse. 1982. The cooperative study of sickle cell disease: review of study design and objectives. *Am.J.Pediatr.Hematol.Oncol.* 4:197-201.

21. Marrades, R. M., O. Diaz, J. Roca, J. M. Campistol, J. V. Torregrosa, J. A. Barbera, A. Cobos, M. A. Felez, and R. Rodriguez-Roisin. 1997. Adjustment of DLCO for hemoglobin concentration. *Am.J.Respir.Crit Care Med.* 155:236-241.

22. Crapo, R. O., A. H. Morris, and R. M. Gardner. 1981. Reference spirometric values using techniques and equipment that meet ATS recommendations. *Am.Rev.Respir.Dis.* 123:659-664.

23. Crapo, R. O. and A. H. Morris. 1981. Standardized single breath normal values for carbon monoxide diffusing capacity. *Am.Rev.Respir.Dis.* 123:185-189.

24. Roberts, C. M., K. D. MacRae, A. J. Winning, L. Adams, and W. A. Seed. 1991. Reference values and prediction equations for normal lung function in a non-smoking white urban population. *Thorax* 46:643-650.

25. Lung function testing: selection of reference values and interpretative strategies. American Thoracic Society. 1991. *Am.Rev.Respir.Dis.* 144:1202-1218.

26. Ballas SK, Marcolina MJ. 2006. Hyperhemolysis during the evolution of uncomplicated acute painful episodes in patients with sickle cell anemia. *Transfusion* 46:105-110.

27. Guasch A, Cua M Mitch WE. Early detection and the course of glomerular injury in patients with sickle cell anemia. 1996. Kidney Int 49, 786-791.

28. Gladwin, M. T. and G. J. Kato. 2005. Cardiopulmonary complications of sickle cell disease: role of nitric oxide and hemolytic anemia. *Hematology.(Am.Soc.Hematol.Educ.Program.)*51-57.

29. Aaron, S. D., R. E. Dales, and P. Cardinal. 1999. How accurate is spirometry at predicting restrictive pulmonary impairment? *Chest* 115:869-873.

30. Leong CS, Stark P. 1998. Thoracic manifestations of sickle cell disease. *J Thorac Imaging* 13:128-134.

31. Akinola NO, Stevens SM, Franklin IM, Nash GB, Stuart J. Subclinical ischaemic episodes during the steady state of sickle cell anaemia. J Clin Pathol 1992:902-906.

32. Milner PF, J. C. B. G. 1994. Bone and Joint Disease. In H. R. M. N. S. M. Embury SH, editor Sickle Cell Disease: Basic Principles and Clinical Practice Raven, New York. 645-661.

33. Silva CM, Viana MB. 2002. Growth deficits in children with sickle cell disease. *Arch Med Res* 33:308-312.

34. Gourgoulianis KI, Molyvdas PA. 2002. Growth of thorax in genetic anemias: an index of deficit (letter). *Arch Med Res* 33:600.

35. Steinberg, M. H. 2005. Predicting clinical severity in sickle cell anaemia. *Br.J.Haematol.* 129:465-481.

36. Flaherty KR, Martinez FJ. 2000. The role of pulmonary function testing in pulmonary fibrosis. *Curr Opin Pulm Med* 6:404-410.

37. Steen, V. and T. A. Medsger, Jr. 2003. Predictors of isolated pulmonary hypertension in patients with systemic sclerosis and limited cutaneous involvement. *Arthritis Rheum.* 48:516-522.

38. Kato, G. J., V. R. McGowan, R. F. Machado, J. A. Little, V. J. Taylor, C. R. Morris, J. S. Nichols, X. Wang, M. Poljakovic, S. M. Morris Jr, and M. T. Gladwin. *Blood* 2005. Lactate dehydrogenase as a biomarker of hemolysis-associated nitric oxide resistance, priapism, leg ulceration, pulmonary hypertension and death in patients with sickle cell disease.

Figure Legends:

Figure 1: Graphic representation of adjusted D_LCO compared with age (yrs). D_LCO was adjusted for hemoglobin concentration prior to this analysis. There was a decrease in D_LCO associated with increasing age. D_LCO – Diffusion capacity for carbon monoxide.

Figure 2: Graphic representation of unadjusted D_LCO compared with serum hemoglobin concentrations (mg/dl). There was a trend towards a decreased D_LCO with increasing anemia. D_LCO – Diffusion capacity for carbon monoxide.

Table 1: Patient Characteristics

	All Patients (n = 310)
Clinical and Demographic Characteristics	
Age (yrs)	30.72 ± 10.34
% Males	41
Height (cm)	170.07 ± 9.56
Weight (kg)	61.28 ± 12.20
BMI	21.25 ± 4.22
History of ACS (%)	37
Episode of ACS during study (%)	38
ACS episodes during study	2.48 ± 1.23
History of Chronic Transfusions (%)	17
Summary of Laboratory Values	
White Blood Cell Count (WBC) (cells/l/mm^3)	11.75 ± 2.64
Hemoglobin (Hb) (g/dL)	8.25 ± 1.44
Platelet Count (x 100,000/mL)	410.96 ± 113.46
Hematocrit (%)	24.76 ± 3.91
Blood Urea Nitrogen (BUN) (mg/dL)	10.00 ± 6.63
Creatinine (mg/dL)	0.87 ± 0.60
Lactate Dehydrogenase (LDH) (units/L)	497.39 ± 166.67
Bilirubin (mg/dL)	3.62 ± 2.13
Aspartate Aminotransferase (AST) (units/L)	47.24 ± 23.93
Alanine Aminotransferase (ALT) (units/L)	44.90 ± 52.31

Table 2: Summary of PFT results

	All Patients (n = 310)
Summary of PFT Results	
FEV_1	
Median	82.80
Mean ± sd	83.03 ± 16.06
FVC	
Median	83.62
Mean ± sd	84.37 ± 16.01
FEV_1/FVC (%)	
Median	98.61
Mean ± sd	98.36 ± 9.15
TLC	
Median	69.79
Mean ± sd	70.20 ± 14.69
RV	
Median	78.04
Mean ± sd	88.60 ± 60.88
D_LCO	
Median	53.74
Mean ± sd	56.57 ± 20.11
Adjusted D_LCO[1]	
Median	61.74
Mean ± sd	64.54 ± 19.93
Sub-classification Based on PFTs	
Normal	31 (10)
Isolated Low D_LCO	40 (13)
Mixed O/R	5 (2)
Obstructive	4 (1)
Restrictive	230 (74)

[1]Adjusted for hemoglobin concentration

Table 3: Comparison of PFT Results According to History of ACS

	History of ACS (n=221)	No History of ACS (n=89)	p value
PFT Results			
TLC (%)	69.17 ± 1.01	72.83 ± 1.62	0.06
D_LCO (%)	55.32 ± 1.43	59.94 ± 2.36	0.10
Adjusted D_LCO (%)	63.32 ± 1.43	67.81 ± 2.34	0.10
Sub-classification Based on PFTs			
Normal n (%)	20 (9)	11 (12)	
Isolated Low D_LCO	27 (12)	13 (15)	
Mixed O/R	3 (1)	2 (2)	0.6073
Obstructive	2 (1)	2 (2)	
Restrictive	169 (76)	61 (69)	

Table 4: Association of laboratory measures with sub-classifications of PFT results.

	Significant variables (α = 0.1)	P value	Case mean	Control Mean
Low D_LCO vs Normal D_LCO			**(n = 40)**	**(n = 64)**
Unadjusted	Creatinine	0.07	1.11	0.8
	BUN	0.0002	11.9	7.7
	ALT	0.06	55.8	33.1
Age-adjusted	Creatinine	0.07	1.11	0.8
	BUN	0.05	10.9	8.3
	Platelets	0.05	441.0	391.9
	ALT	0.08	59.2	31.1
Restrictive disease vs Normal PFTs			**(n = 230)**	**(n = 31)**
Unadjusted	Hb	0.08	8.3	8.7
	Creatinine	0.08	0.9	0.7
	BUN	0.07	9.9	7.7
	HCT	0.05	24.7	26.2
Age-adjusted	Hb	0.08	8.3	8.7
	WBC	0.06	0.9	0.7

Fig. 1

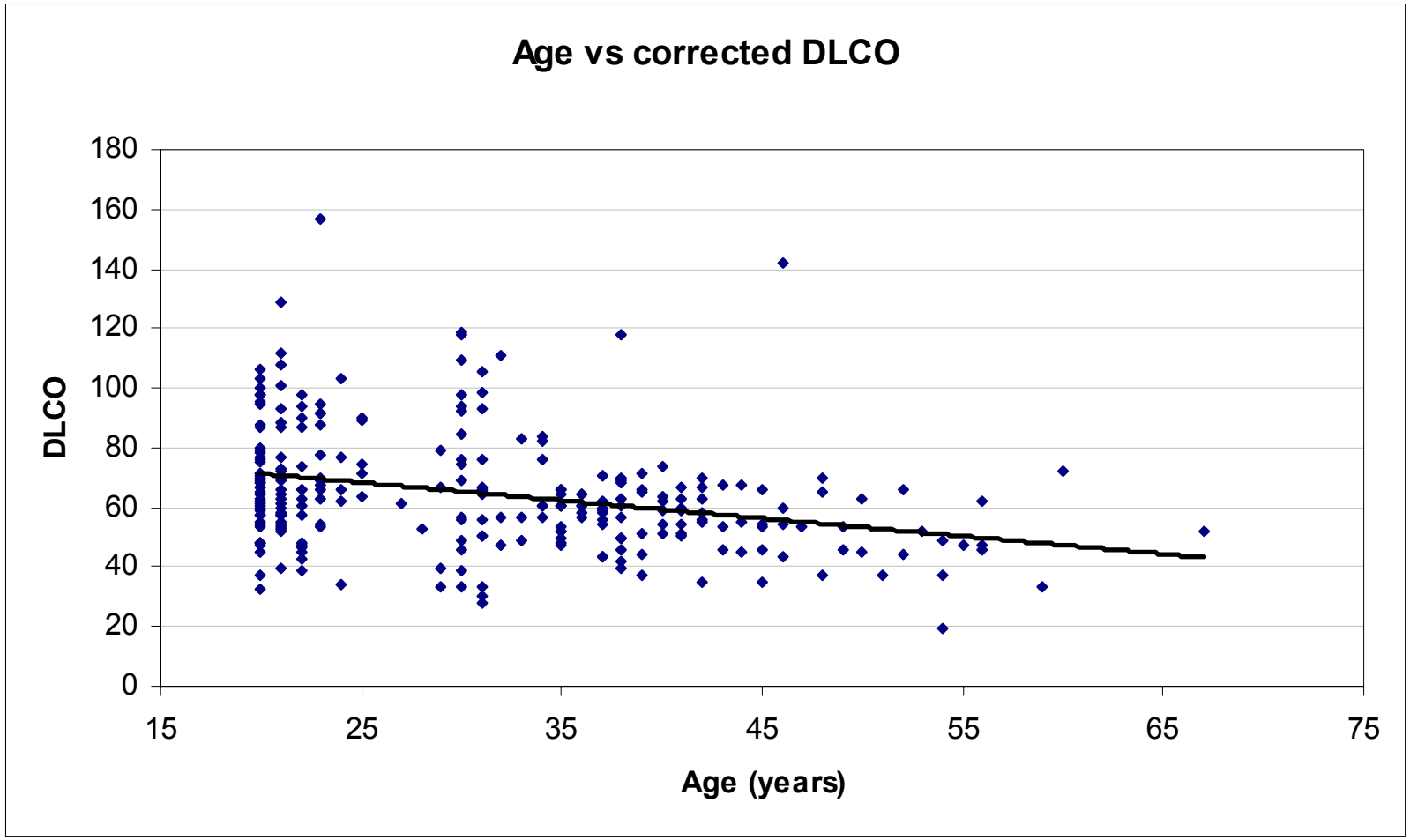
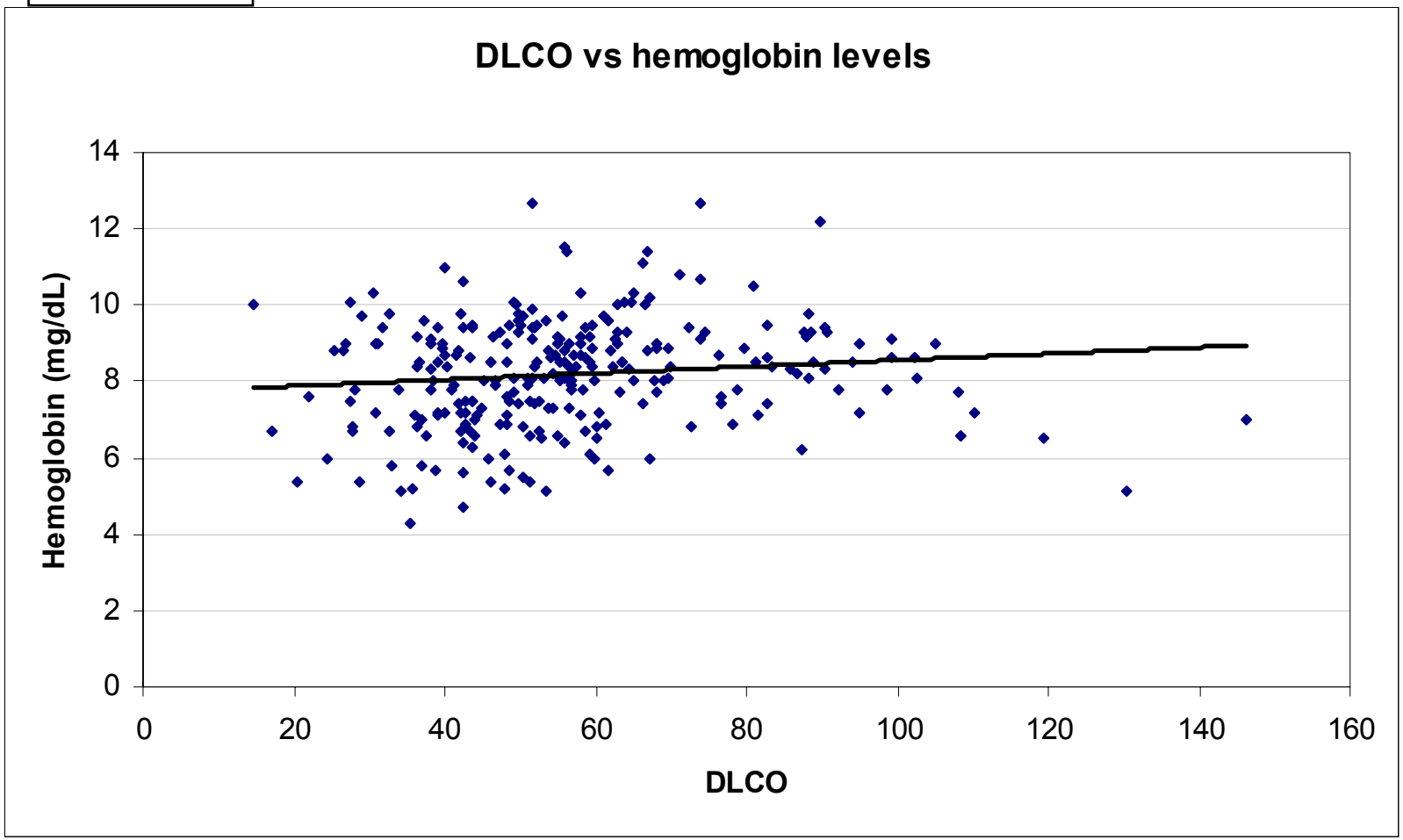

Age vs corrected DLCO
DLCO
180
160
140
120
100
80
60
40
20
0
15
25
35
45
55
65
75
Age (years)

Fig. 2


DLCO vs hemoglobin levels
Hemoglobin (mg/dL)
14
12
10
8
6
4
2
0
0
20
40
60
80
100
120
140
160
DLCO