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Biomarkers of Clinical Severity in Treated and Untreated Sickle Cell Disease: A Comparison by Genotypes of a Single Center Cohort and African Americans in the NHANES Study

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Abstract

Background.—Hemolysis and vaso-occlusion underlie multi-organ system complications in sickle cell disease (SCD).

Methods.—We assessed real-world biomarkers in University of Illinois adult SCD patients, categorized as severe (HbSS/Sβ^0-thalassemia; n=342) or mild (HbSC/Sβ^+-thalassemia; n=100) genotypes and stratified according to treatment. African-American controls from the National Health and Nutrition Examination Survey (NHANES) were matched with each genotype category.

Results.—Most measures of hemolysis, anemia, inflammation, and function of kidneys, liver and lungs differed markedly in untreated severe genotype patients compared to NHANES controls. These same biomarkers were significantly closer to the NHANES control range in untreated mild versus severe genotype patients, but they were not improved in severe genotype patients receiving treatment with hydroxyurea or blood transfusions, except that hemoglobin and HbF were higher with hydroxyurea. Systolic blood pressures did not differ among the SCD and NHANES groups, but diastolic pressures were higher in mild genotype patients. Ferritin in severe genotype patients on chronic transfusions was 50-fold higher than NHANES controls.

Discussion.—The cross-sectional real-world biomarkers of patients on hydroxyurea or transfusions were not markedly improved compared to untreated patients. This may be due partly

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Authorship

FN drafted the manuscript and performed the statistical analysis. XZ performed the statistical analyses and selected the NHANES subjects included in the analyses. RFM, SLS and VRG contributed to recruiting and clinically studying the UIC SCD cohort. All authors contributed to the concept and design of the study. All authors critically reviewed and approved the manuscript.

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to poor compliance or more severe disease. Our findings highlight the need for more effective treatments.

Keywords

Sickle cell disease; SCD phenotype; SCD genotype; biomarker; NHANES

Introduction

Sickle cell disease (SCD) is characterized by polymerization of deoxygenated hemoglobin S, which induces erythrocyte deformation, hemolytic anemia, and microvascular vaso-occlusion.[1–3,6] The most frequent genotype for SCD is the homozygous S mutation (Hb SS), constituting about 70% of SCD patients. Less frequent genotypes include compound heterozygous associations of HbS and HbC (Hb SC), or HbS and β-thalassemia (Hb Sβ-thalassemia).[7] Broadly speaking, SCD patients can be categorized into mild (Hb SC and Hb $S\beta^+$-thalassemia) or severe (Hb SS and Hb $S\beta^0$-thalassemia) β-globin groups,[8,43] but these genotype groups do not completely account for the clinical course of the disease.[4,5,9] Even within these groups, clinical severity varies remarkably, ranging from a barely perceptible clinical disease course to severely debilitating illness resulting in a host of complications.[1–3] Genetic, epigenetic, environmental and therapeutic factors contribute to this phenotypic variability.[5,11] Laboratory measures can be a powerful supplement to genetic data in predicting morbidity and mortality.[4,7,21] The aim of this study was to compare routine laboratory and imaging biomarkers by the two broad SCD genotype severity categories, stratified according to disease modifying treatment, and matched by age, race and gender to a non-SCD National Health and Nutrition Examination Survey (NHANES) cohort. The study was done using real-world data from a single center involving an adult cohort of SCD patients.

Methods

We cross-sectionally analyzed 442 adult SCD patients receiving medical care at the University of Illinois at Chicago (UIC) between 2009–2017. The protocol was approved by the UIC Institutional Review Board. Clinical data including demographics, sex, race/ethnicity, past medical history, vital signs, laboratory values, radiographic results and echocardiogram were extracted from the Cerner Power Chart electronic health records (EHRs). Baseline laboratory, blood pressure, and anthropometric results were recorded for each patient using values from outpatient visits. Patients were at steady state, not in crisis, and at baseline level of pain. SCD genotype was determined by high-performance liquid chromatography (HPLC) fractionation of hemoglobin or by hemoglobin electrophoresis. SCD patients were categorized into 2 severity subgroups: mild (Hb SC and Hb $S\beta^+$-thalassemia; n=100) and severe (Hb SS and Hb $S\beta^0$-thalassemia; n=342) β-globin genotype groups. For a comparison to the background population, we selected African Americans from National Health and Nutrition Examination Survey (NHANES) 2009–2012 data and matched 684 NHANES controls to 342 severe SCD genotype patients and 200 additional NHANES controls to 100 mild SCD genotype patients in a 2:1 ratio by age and gender. We categorized SCD individuals in sub-groups of i) not on therapy, ii) receiving hydroxyurea, or

iii) on chronic red blood cell transfusions with or without hydroxyurea therapy. Individuals classified as not on therapy had not been on hydroxyurea or routine RBC transfusions in the three months preceding data collection. SCD individuals not on hydroxyurea therapy did not qualify for hydroxyurea because of non-severe disease, were intolerant to hydroxyurea, or were non-compliant with therapy. Individuals on chronic red blood cell transfusions were either on regular automated exchange transfusion or simple transfusion. Transfusion interval ranged from 4–8 weeks. For between-group statistical comparisons, we used Wilcoxon's rank sum test for continuous variables and Fisher's exact test for categorical variables. We indicated P-values that were significant after the Bonferroni correction for multiple comparisons. The associations of HbF% with hemoglobin concentration and with white blood cell count were analyzed with linear regression, with HbF% variables square root transformed. Statistical analyses were carried out using SYSTAT 13, Systat Software, Inc.

Results

Characteristics of study participants.

A greater proportion of the 442 SCD participants had a severe genotype (77.4%) than a mild genotype (22.6%). Of the 342 severe genotype patients, 121 (35.4%) were not on therapy, 153 (44.7%) were on hydroxyurea and 68 (19.9%) were on chronic red blood cell transfusions; 25 of these chronic transfusion patients were also receiving hydroxyurea. In contrast, 70% of the 100 mild genotype patients were not on therapy, whereas 30% were on hydroxyurea; three of these hydroxyurea patients were also on chronic transfusion (Tables 1, 2). The median ages of severe genotype patients ranged from 28–31 years according to treatment category; median ages were 38 and 33 years in the mild genotype groups. Over 50% of the participants in each treatment category were females, except for a slight predominance of males in severe genotype patients on chronic transfusions (Table 2).

Biomarkers of SCD patients not on disease modifying therapy compared to NHANES controls.

When compared with their respective NHANES controls, non-treated patients with either severe or mild SCD genotypes had lower hemoglobin concentrations: 13.5 g/dl (NHANES) vs 8.4 g/dl in severe genotype patients; 13.1g/dl (NHANES) vs 11.3 g/dl in mild genotype patients. White blood cell (WBC) counts, lactic acid dehydrogenase (LDH) levels, total bilirubin concentrations and urine albumin to creatinine ratios were strikingly higher in both severe and mild genotype patients than in their respective NHANES controls ($P<0.0001$ for all comparisons, Table 1, Figure 2). On the other hand, median platelet counts, ferritin levels, and values for aspartate amino transferase (AST), alkaline phosphatase, forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC) were higher in severe genotype patients when compared to NHANES controls ($P<0.0001$), but not in mild SCD patients compared to controls (Table 1, Figure 3). The median serum ferritin level was six times higher in untreated severe SCD genotype patients compared to NHANES controls and 50-fold higher in severe genotype patients on chronic transfusions (Tables 1, 2). Serum creatinine was lower and estimated glomerular filtration rate (calculated using the Chronic Kidney Disease Epidemiology Collaboration formula)[42] was higher in severe SCD genotypes compared to NHANES control individuals (Table 1, Figure 2) suggesting

hyperfiltration, but untreated mild SCD genotype patients had similar GFR to NHANES controls. There was no observed difference in systolic blood pressure (SBP) between untreated SCD patients and their NHANES controls, regardless of genotype or blood pressure treatment status. However, untreated mild SCD genotype participants had higher diastolic blood pressure than NHANES controls regardless of antihypertensive therapy ($p<0.0001$; Table 1).

Comparison of biomarkers among SCD patients according to genotype and treatment categories (Table 2).

Markers of hemolysis, anemia and hypoxia.—Compared to the untreated severe SCD genotype group, markers of hemolysis including reticulocytes, LDH, indirect bilirubin and AST were lower in the mild genotype groups regardless of treatment, but they were not lower in the severe genotype patients receiving hydroxyurea or chronic transfusions (Figure 1). Also compared to the untreated severe genotype group, the median hemoglobin concentration was higher in the mild SCD genotype groups and in the severe SCD genotype patients treated with hydroxyurea, but not in the severe genotype patients receiving chronic transfusions (Table 2). HbF% was higher in individuals with severe genotype on hydroxyurea therapy compared to the untreated group and had a strong positive correlation with hemoglobin level ($p<0.0001$; Figure 4). HbF% was also higher in untreated severe SCD genotype patients than mild genotypes regardless of treatment status. The median oxygen saturation by pulse oximetry was higher in the mild SCD genotype groups than the untreated or hydroxyurea-treated severe SCD genotype groups (Table 2).

Markers of inflammation and iron overload.—Compared to the untreated severe SCD group, the WBC count was lower in untreated mild genotype patients but not in severe genotype patients receiving disease modifying therapy (Table 2, Figure 2). There was a strong negative correlation between total WBC count and HbF% in individuals with severe genotype on hydroxyurea treatment ($p<0.0001$, Figure 4). Platelet counts were lower in the mild SCD genotype groups but not in the severe SCD groups being treated with hydroxyurea or chronic transfusions (Table 2). The median ferritin was eight times higher in the severe SCD subgroup on chronic transfusions than the untreated severe SCD group (Table 2, Figure 2).

Markers of cardiopulmonary function.—Systolic blood pressures did not differ among the SCD groups, but diastolic blood pressures were higher in the mild SCD genotype patients than severe SCD patients regardless of antihypertensive therapy. In general, echocardiographic and pulmonary function parameters did not differ significantly in the SCD groups based on genotype and treatment, except that untreated mild SCD patients had greater FEV1 and FVC than untreated severe SCD patients (Table 2, Figure 3).

Markers of renal function.—Compared to the untreated severe SCD genotype group, severe genotype patients receiving hydroxyurea or transfusion did not have a difference in serum creatinine, eGFR or urine albumin to creatinine ratio. On the other hand, untreated mild SCD patients had higher creatinine and lower GFR values that were still within the normal range, suggesting protection from potentially harmful hyperfiltration. In keeping

with this interpretation, there was no difference in urinary albumin-to-creatinine ratio in the severe SCD patients on treatment compared to untreated severe genotype patients, but it was markedly lower in mild genotype patients, notwithstanding treatment status of the mild SCD genotype group. (Tables 2, Figure 2).

Hepatic markers.—Alanine amino transferase (ALT) was higher in the severe SCD subgroup on chronic transfusion when compared to untreated severe SCD patients, likely reflecting predisposition to liver disease with repeated blood transfusions and iron overload. Direct bilirubin, which is elevated if bilirubin conjugation to glucuronic acid is impaired, was lower in the mild SCD genotype groups versus the untreated severe genotype group (Table 2).

Discussion

Here we provide a profile of adult SCD patients compared to NHANES controls using clinical biomarkers obtained during routine care. We present biomarkers that highlight organ system dysfunction in predominantly young adults with SCD, stratifying patients according to mild versus severe SCD genotypes and according to treatment status at the time of data collection. We assessed how clinical markers vary across genotype and treatment groups.

Individuals with severe SCD genotypes generally have chronically low hemoglobin, which is indicative of chronic hemolysis and reduced red cell survival of typically 15–17 days.[12,14,19] Individuals with mild genotypes, though having lower hemoglobin than the background population, have less chronic hemolysis and thus can maintain higher hemoglobin than severe genotype patients. As expected, anemia was more pronounced with the severe and mild SCD genotypes in comparison to NHANES controls in this study. Low hemoglobin is associated with poor prognosis; it correlates with increased risk of high tricuspid regurgitation velocity (TRV), hemorrhagic stroke, ischemic stroke, and premature death.[16,20,22,23] Measures of hemolysis, such as LDH and AST released from erythrocytes to plasma during red blood cell breakdown,[21,23] were markedly higher in SCD patients compared to NHANES controls, regardless of genotype. The WBC count, often increased in SCD due to chronic inflammation and hyposplenism,[13,1424] was markedly elevated in SCD patients versus NHANES controls and was higher in severe versus mild genotype patients.[24] Steady-state elevation in WBC count is associated with adverse outcomes, including increased occurrence of pain crisis, increased hemorrhagic stroke risk, and early mortality.[13–17] In contrast to prior studies,[47] we did not observe lower systolic and diastolic blood pressures in severe genotype SCD patients versus NHANES controls, and, more to be expected we did not observe lower blood pressures in mild genotype patients either.[47] However, diastolic blood pressures were actually higher in mild genotype patients compared to NHANES controls and severe genotype patients, and we do not have a good explanation for this.

Studies of pulmonary function in the SCD population have yielded a spectrum of abnormalities, including restrictive lung disease, abnormal diffusion capacity for carbon monoxide (DL_{CO}), obstructive disease, and hypoxemia.[31–34] Our study showed markedly lower percent predicted FEV1 and FVC in severe but not mild SCD genotypes compared to

matched NHANES controls. Risk for developing pulmonary hypertension in SCD correlates with steady-state severity of hemolysis and anemia.[20,26] Whereas our study demonstrated difference in hemolysis markers between the severe and mild genotype groups, it failed to show a difference in the TRV, a noninvasive marker of systolic pulmonary artery pressure.[27,29,30] Renal dysfunction in SCD is caused by both vaso-occlusive phenomena and hemolysis-related vasculopathy.[35,36] When acute or chronic hemolysis overwhelms endogenous scavengers of heme products such as haptoglobin and hemopexin, the kidneys are exposed to the injurious effects of heme and iron.[35] Amongst biomarkers in routine clinical use, albuminuria appears to have the strongest association with early sickle cell nephropathy.[36] In our analysis, eGFR was significantly increased in severe SCD genotypes compared to NHANES controls, possibly reflecting the effect of prostaglandins derived from medullary ischemia, tubular secretion of creatinine into the urine, low muscle mass, and/or increased cardiac output of the patients.[36] At the same time, the urinary albumin to creatinine ratio was significantly elevated in severe SCD phenotypes versus NHANES controls, reflecting the predisposition to chronic kidney disease despite the higher eGFR.[37] SCD causes a variety of pathologies in the liver, but in addition, therapy of SCD can cause liver injury from transfusion related iron overload and viral hepatitis.[38–39] The frequency of cirrhosis at autopsy has been reported as high as 11–14%.[39–41] Untreated severe SCD patients in this study had higher ALT and alkaline phosphatase (ALP) than the NHANES controls.

Hydroxyurea, an oral therapeutic agent,[18, 46] has multiple physiological effects, including increasing HbF expression in most individuals with severe sickle genotype[18] and decreasing leukocyte count.[45] Hydroxyurea reduces the frequency of pain crisis and hospitalization and has a good safety profile with close monitoring.[18] In most affluent countries, up to 63% of severe sickle genotype patients are on hydroxyurea.[45] Consistently, 65% of severe genotype patients in this cohort were either on hydroxyurea or chronic RBC transfusions. In our analysis, only hemoglobin and HbF% were significantly different in severe genotype patients according to hydroxyurea treatment, showing a rise with treatment. In these patients, hemoglobin correlated positively with the HBF% (Figure 4), consistent with prior studies.[18] HbF prevents the polymerization of HbS under deoxygenated conditions and is a major marker and modulator of disease severity. The importance of HbF is demonstrated by the mild clinical course of individuals who are compound heterozygous for HbS and hereditary persistence of fetal hemoglobin.[3,10] However, some patients on hydroxyurea do not have a beneficial response, usually because of poor adherence to treatment but possibly because of pharmacogenomic reasons.[5,7,45] As with previous studies,[45] individuals with severe SCD genotype receiving hydroxyurea therapy had significantly lower total WBC count with rising HbF% (Figures 2, 4). Since hydroxyurea is a myelosuppressive agent that lowers WBC count, this observation is consistent with a dose-related effect of hydroxyurea in raising the HbF% and preventing clinical complications in SCD.[18]

Chronic blood transfusion therapy is often achieved by periodic simple erythrocyte transfusion or periodic automated red cell exchange transfusions (ARCET). The goal of this therapy is to decrease the number of circulating sickle erythrocytes, thereby improving microvascular flow and reducing endothelial injury and inflammatory damage.[25,44, 45] However adverse effects, including iron overload, alloimmunization and hemolytic

transfusion reactions, limit its use and potential benefits.[45] Severe genotype patients in our analysis received transfusion therapy as secondary prophylaxis for stroke,[16] severe end-organ damage, intractable pain crisis, pulmonary hypertension and diastolic heart failure[25], which are important risk factors for death.[20,26,27,28] Other than levels of HbF%, ferritin, ALT, and ALP, there was no significant difference in the biomarkers of severe SCD patients on chronic transfusion compared to untreated severe SCD. HbF% was reduced in patients undergoing chronic transfusion, possibly because most of our patients receiving this modality were on ARCET, which removes sickle erythrocytes relatively rich in HbF and replaces them with HbA erythrocytes poor in HbF. The increased levels of ferritin, ALT and ALP in chronic transfusion patients likely reflect predisposition to liver disease with repeated blood transfusion and iron overload. SCD patients receiving chronic transfusion therapy are monitored for evidence of iron overload using serum ferritin, which correlates with hepatic iron content and total blood transfusion burden. A serum ferritin level greater than 1,000 ng/mL is considered evidence of iron overload but is unreliable for evaluating iron status independently as it remains elevated in SCD because of chronic inflammation.[39] Levels were more prominently elevated in severe than mild SCD genotype patients in this study even if not receiving chronic transfusion therapy, possibly due to greater episodic transfusions in the past. In severe SCD genotype patients on chronic transfusion therapy, median ferritin reached 8 times higher than untreated severe SCD, likely reflecting predominantly a transfusional increase in iron stores in these patients.

This study is limited by its cross-sectional design. Clinical markers were obtained as part of standard of care during routine outpatient visits. Thus, clinical markers that are not obtained routinely in SCD patients were likely obtained because of suspicion for additionally pathology, therefore selecting participants with skewed numbers. For instance, lung spirometry was obtained in 15% and 24% of mild and severe SCD phenotypes respectively. Additionally, many clinical markers of organ dysfunction were unavailable for the NHANES cohort. The untreated severe SCD genotype patients may be intrinsically milder than the ones that go on to treatment, so the benefit of the treatment in Table 2 may be masked by biomarkers being a lot more deviated from normal before they started treatment. Given our data set we are not able to determine exactly how compliant patients are with therapy. But the HbF% being significantly higher in the severe SCD genotype on hydroxyurea suggests there is a substantial amount of compliance with the treatment programs.

In conclusion, our analysis illustrates the differences in phenotype of SCD according to genotype severity categories in real world data collected during routine patient care. It also shows that the cross-sectional phenotype of patients on hydroxyurea or blood transfusions was not markedly improved from untreated patients, except for hemoglobin concentration and HbF percent with hydroxyurea. This observation may be due to poor compliance with therapy or more severe disease phenotype before starting therapy. Nevertheless, our findings highlight the need for ongoing research to develop new, more effective treatments for SCD. The clinical measures are also potential outcome variables for planning and conducting observational and therapeutic trials in the real-world setting.

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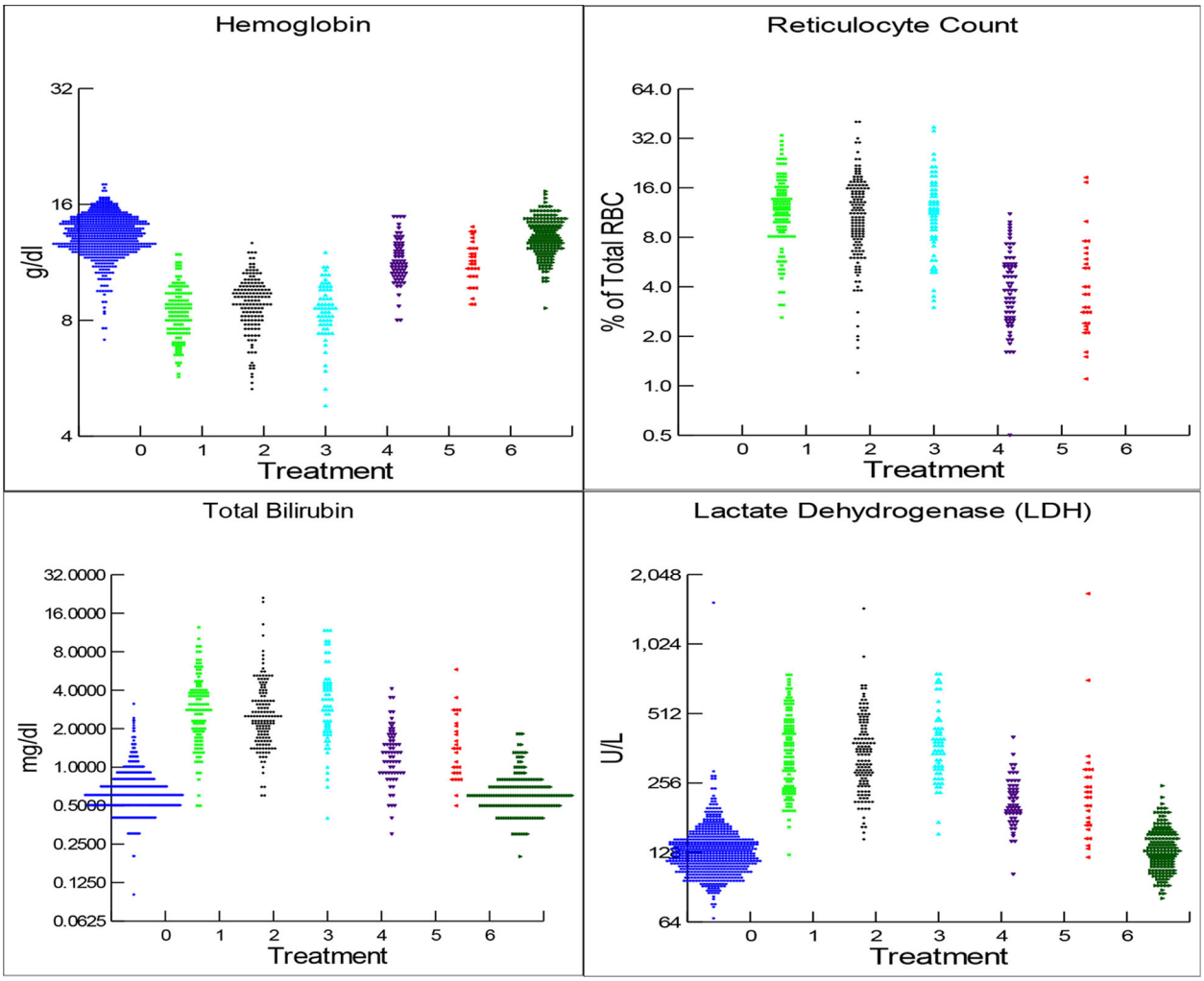

Figure 1.

Hemoglobin, WBC, total bilirubin and LDH subdivided by treatment status and SCD phenotype.

NHANES 1 and NHANES 2 are controls for severe and mild SCD genotypes, respectively.

Y-axis values are plotted to log base 2 to accommodate outliers.

0 – NHANES 1

1 – Severe SCD – no therapy

2 – Severe SCD on hydroxyurea

3 – Severe SCD on chronic RBC transfusion

4 – Mild SCD – no therapy

5 – Mild SCD on Hydroxyurea

6 – NHANES 2

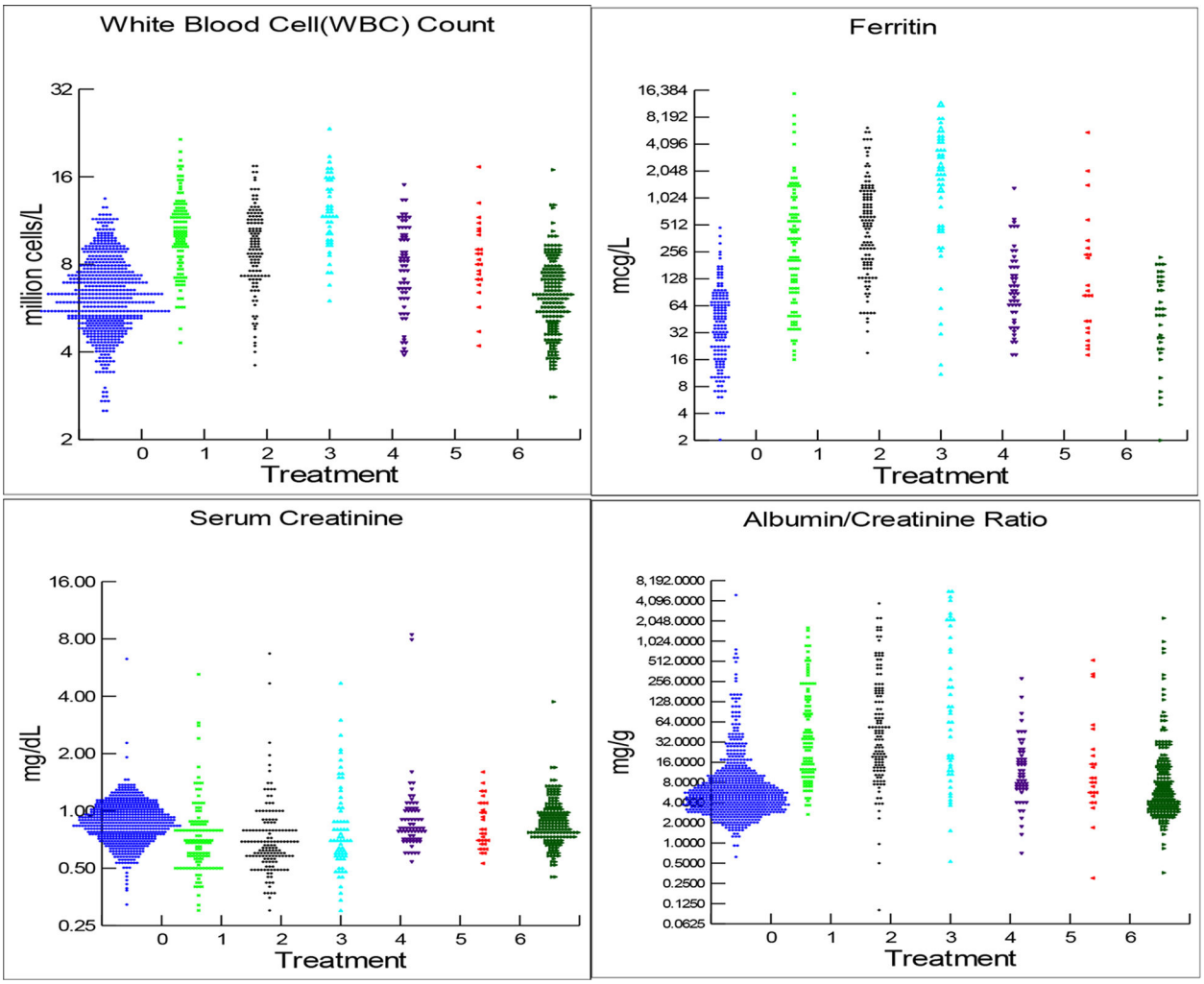

Figure 2.

Ferritin, serum creatinine, FEV1, ACR, subdivided by treatment status and SCD phenotype. NHANES 1 and NHANES 2 are controls for severe and mild SCD genotypes, respectively. Y-axis values are plotted to log base 2 to accommodate outliers.

0 – NHANES 1

1 – Severe SCD – no therapy

2 – Severe SCD on hydroxyurea

3 – Severe SCD on chronic RBC transfusion

4 – Mild SCD – no therapy

5 – Mild SCD on Hydroxyurea

6 – NHANES 2

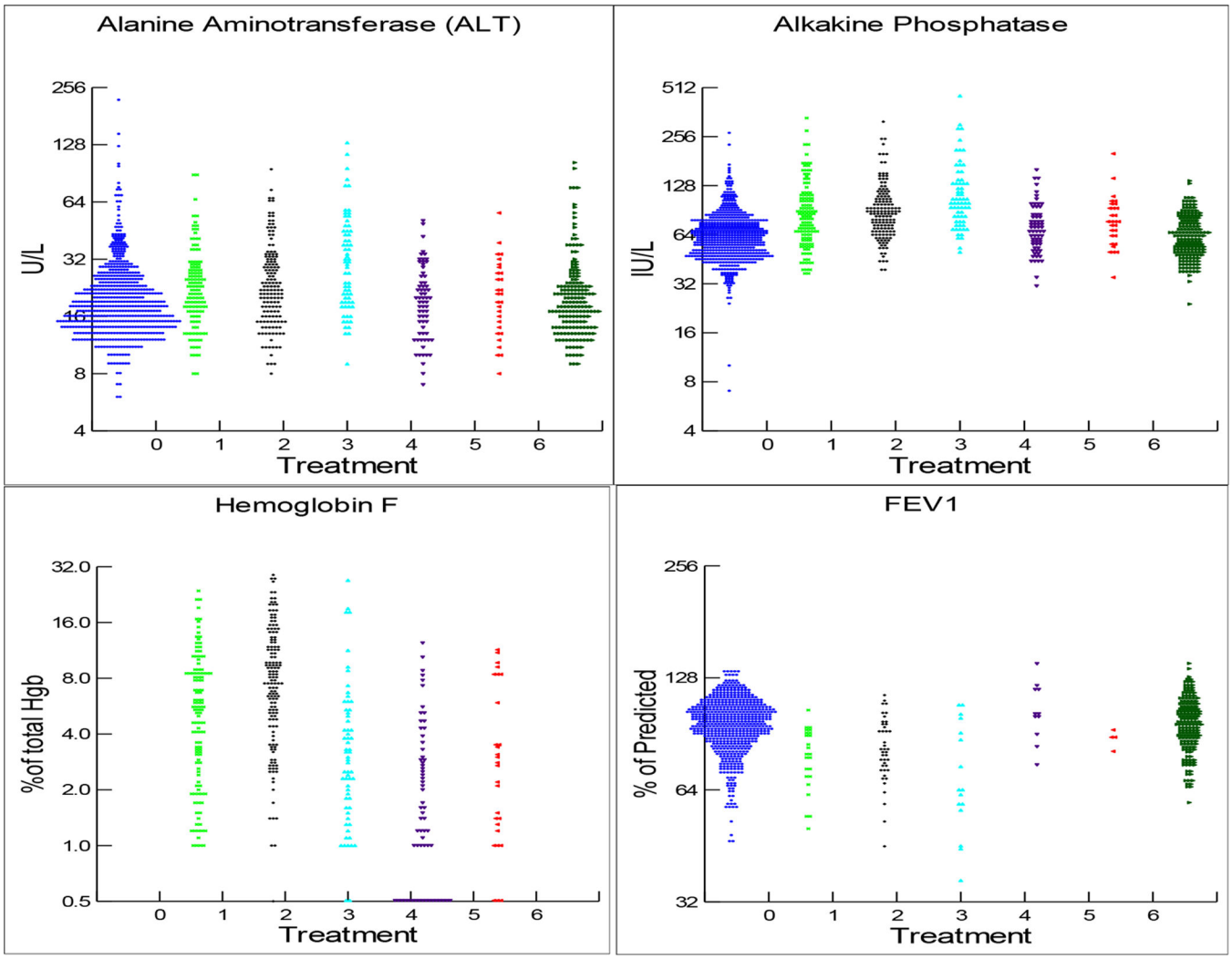

Figure 3.

ALT, ALP, HBF, reticulocyte count, subdivided by treatment status and SCD phenotype. NHANES 1 and NHANES 2 are controls for severe and mild SCD genotypes, respectively. Y-axis values are plotted to log base 2 to accommodate outliers.

0 – NHANES 1

1 – Severe SCD – no therapy

2 – Severe SCD on hydroxyurea

3 – Severe SCD on chronic RBC transfusion

4 – Mild SCD – no therapy

5 – Mild SCD on Hydroxyurea

6 – NHANES 2

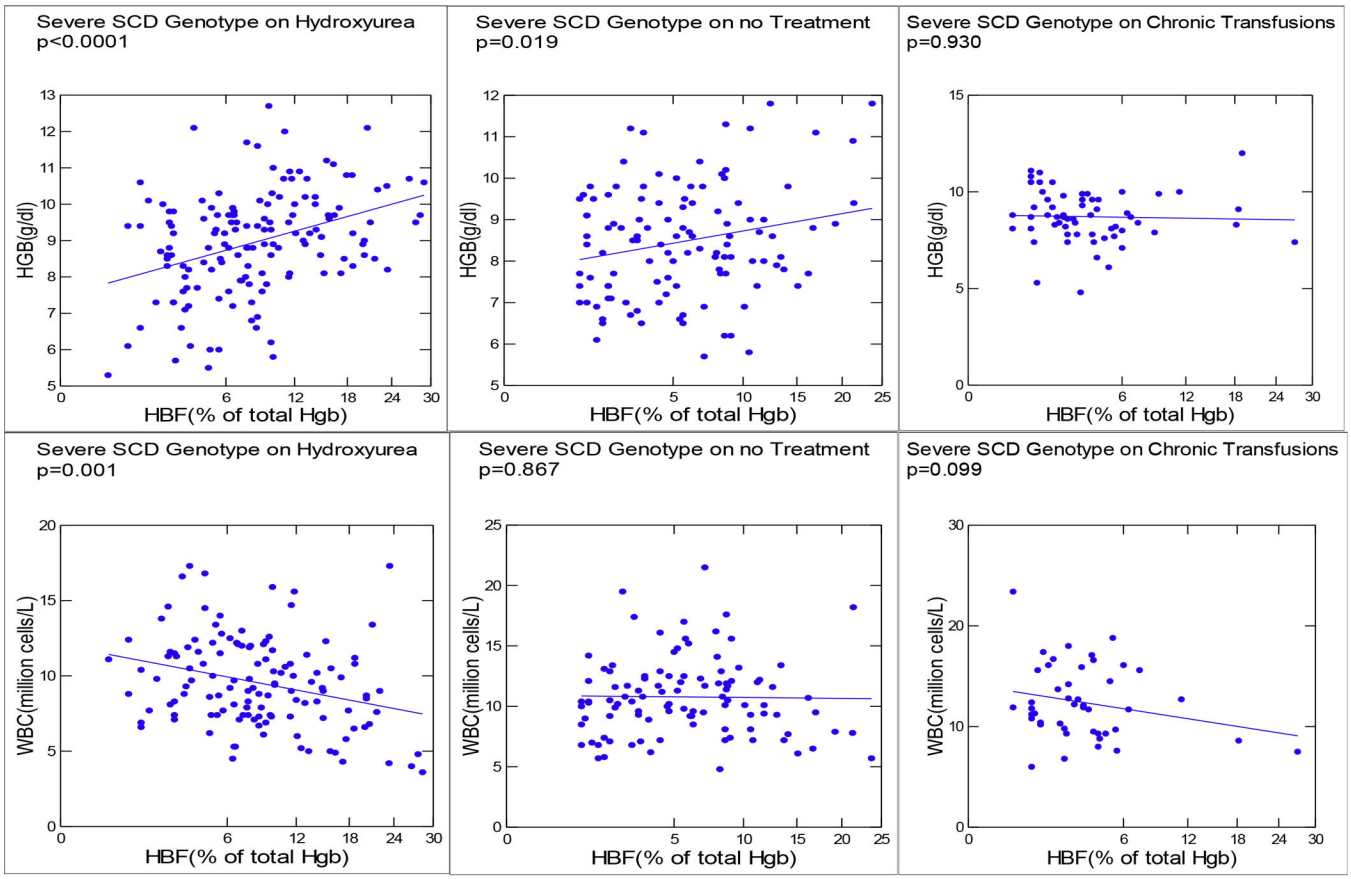

Figure 4.

In individuals with severe genotype receiving hydroxyurea therapy, HBF% correlated positively with the hemoglobin level and inversely with WBC count.

Table 1.

Clinical markers in SCD patients without disease-modifying treatment stratified by severity group compared to NHANES controls matched by age and sex. Results in median (IQR) or no. (%)

	NHANES controls for severe SCD genotypes			Severe SCD: No Treatment			NHANES controls for mild SCD genotypes			Mild SCD: No Treatment		
marker	**n**	**result**		**n**	**result**	**p***	**n**	**result**		**n**	**result**	**p***
Demographics												
Age(years)	684	30 (23–41)		121	31(25–41)	0.350	200	36(24–47)		70	38(25–49)	0.526
Female gender, n (%)	684	378 (55.3%)		121	73(60.3%)	0.301	200	132(66.0%)		70	46(65.7%)	0.965
Hemolysis, Anemia												
Hemoglobin (g/dL)	615	13.5(12.4–14.5)		116	8.4(7.4–9.4)	<0.0001	182	13.1(12.3–14.4)		70	11.3(10.6–12.5)	<0.0001
Hematocrit (%)	615	40(36.8–43.2)		116	25.2(22.3–28.3)	<0.0001	181	39(36.2–43.1)		70	33.2(30.2–36.6)	<0.0001
Lactic acid dehydrogenase ** (U/L)	602	128(111–146)		110	352(244–470)	<0.0001	177	131(113–154)		58	206.5(189.0–249.0)	<0.0001
Inflammation and Iron Overload												
White blood cell count (1000/uL)	615	6.3(5.1–7.8)		105	10.4(8.5–12.3)	<0.0001	181	6.3(5.0–7.6)		64	8.1(6.1–10.0)	<0.0001
Platelets (1000/uL)	615	235 (199–283)		117	433 (33–528)	<0.0001	181	244(213.8–287.3)		70	258(186–365)	0.443
Serum albumin (g/dL)	602	4.2(3.9–4.4)		113	4.1(3.8–4.4)	0.049	177	4.2(3.9–4.4)		67	4.0(3.8–4.3)	0.015
Ferritin (ng/mL)	150	40 (17–76)		91	264 (78–772)	<0.0001	34	55.5(21–122)		50	93(54–180)	0.010
Cardiopulmonary Function												
Systolic blood pressure (mmHg)	616	118(109–128)		117	119(110–128)	0.855	171	120(110–130)		70	120.5(111–130)	0.436
Diastolic blood pressure (mmHg)	616	70(62–78)		117	70(64–74)	0.318	171	64(70–80)		70	78(70–85)	<0.0001
SBP without treatment(mmHg)	559	116(108–126)		102	116(110–125)	0.782	151	118(108.0–129.5)		59	119(110.3–129.0)	0.400
DBP without treatment(mmHg)	559	70(62–76)		102	70(64–73)	0.408	151	70(62.5–79.5)		59	77(70–81)	<0.0001
FEV1(%Predicted)	589	97.9(88.0–107.5)		24	80.2(69.9–91.3)	<0.0001	170	97.6(86.5–108.1)		11	102.5(92.8–119.7)	0.202
FVC (%Predicted)	589	100(89.7–108.8)		24	86.4(73.9–92.5)	<0.0001	170	99.9(89.9–109.2)		11	103.8(93.0–112.5)	0.336
Renal Function												
Serum creatinine (mg/dL)	602	0.88(0.74–1.03)		116	0.7(0.55–0.86)	<0.0001	177	0.85(0.74–1.00)		69	0.8(0.7–1.0)	0.657
eGFR (mL/min/1.73m2)	602	100 (84–123)		104	138 (116–149)	<0.0001	177	100.5(80.8–119.8)		57	108.3(91.4–131.5)	0.083

	NHANES controls for severe SCD genotypes			Severe SCD: No Treatment			NHANES controls for mild SCD genotypes			Mild SCD: No Treatment		
marker	**n**	**result**		**n**	**result**	**p***	**n**	**result**		**n**	**result**	**p***
Urine ACR (ug/mg)	657	5.4(3.4–9.2)		97	31(12.5–141.9)	<0.0001	187	5.5(3.4–13.8)		56	10.3(7.0–21.3)	0.001
Hepatic Markers												
Total bilirubin **(mg/dL)	601	0.6(0.5–0.8)		113	2.9(1.8–4.0)	<0.0001	177	0.6(0.5–0.8)		67	1.3(0.9–1.7)	<0.0001
Aspartate aminotransferase **(U/L)	602	22(18–27)		113	40(28–52.3)	<0.0001	177	21(18–26)		67	25(19.3–30.0)	0.010
Alanine transaminase (U/L)	602	19(15–26)		113	22(17–29)	0.005	177	18(14.0–23.3)		67	19(13.0–25.5)	0.875
Alkaline phosphatase (U/L)	602	63(50–76)		113	83(61.8–110.5)	<0.0001	177	62(50.0–78.3)		66	68.5(56–85)	0.025

ACR- albumin to creatinine ratio; eGFR- estimated glomerular filtration rate; SBP – systolic blood pressure; DBP – diastolic blood pressure; FEV1 – forced expiratory volume in 1 second; FVC- forced vital capacity. All p-values are tested against NHANES control.

*p-values <0.0024 remain significant after the Bonferroni correction for multiple comparisons.

**These variables can be viewed as hemolytic markers or as hepatic markers.

Table 2.

Clinical markers in SCD patients according to mild versus severe genotype groups and disease-modifying treatment. Results in median and interquartile range unless otherwise indicated. P values represent comparison with the Severe SCD No Treatment group

	Severe SCD- No treatment		Severe SCD- On Hydroxyurea			Severe SCD- On Blood Transfusion+			Mild SCD- No Treatment			Mild SCD- On Hydroxyurea		
Marker	**n**	**result**	**n**	**result**	**P***	**n**	**result**	**P***	**n**	**result**	**P***	**n**	**result**	**P***
Demographics														
Age(years)	121	31(25–41)	153	29 (23–41)	0.271	68	28 (22–40)	0.299	70	38(25–49)	0.022	30	33(22–46)	0.883
Female gender, n (%)	121	73(60.3%)	153	84(54.9%)	0.367	68	32(47.1%)	0.078	70	46(65.7%)	0.459	30	20(66.7%)	0.523
Hemolysis, Anemia, and Hypoxia														
Hemoglobin (g/dL)	116	8.4(7.4–9.4)	152	9.15(8.1–9.9)	0.001	66	8.7(7.8–9.6)	0.234	70	11.3(10.6–12.5)	<0.0001	30	11.4(10.4–12.3)	<0.0001
Hematocrit (%)	116	25.2(22.3–28.3)	152	27.4(24.4–29.4)	0.003	66	25.6(23.1–28.0)	0.645	70	33.2(30.2–36.6)	<0.0001	30	33.4(29.7–36.1)	<0.0001
Lactic acid dehydrogenase ** (U/L)	110	352(244–470)	139	342(261–437)	0.536	59	360(296–439)	0.417	58	207 (189–249)	<0.0001	28	224(169–285)	<0.0001
Reticulocytes (%)	113	12.2(9.0–15.6)	150	11.2(7.5–16.1)	0.273	63	11.6(8.1–15.5)	0.522	68	3.9(2.6–5.6)	<0.0001	29	3.6(2.4–6.0)	<0.0001
Absolute reticulocytes (1000/uL)	114	343(249–420)	150	290(214–414)	0.043	63	315(184–466)	0.416	68	162(116–233)	<0.0001	29	131-98-205)	<0.0001
Indirect bilirubin (mg/dL)	114	2.4(1.4–3.5)	142	1.9(1.3–2.8)	0.079	59	2.7(1.5–4.1)	0.180	62	1.0(0.7–1.5)	<0.0001	28	1.1(0.7–1.6)	<0.0001
Hemoglobin F (% of total Hb)	114	5.5(2.5–8.7)	148	7.9(4.7–12.7)	<0.0001	63	2.9(1.6–5.2)	<0.0001	62	1.6(−0.5–3.3)	<0.0001	28	2.8(1.3–7.2)	0.005
Erythropoietin (U/L)	34	69(40–124)	41	82(60–146)	0.128	16	69-(41–96)	0.662	6	66(29–75)	0.325	7	36(31–51)	0.054
Oxygen saturation (%)	73	96(95–98)	102	97(95–99)	<0.072	47	97(95–98)	0.407	50	99(97–100)	<0.0001	21	98(97–99)	0.003
Inflammation and Iron Overload														
White blood cell count (1000/uL)	105	10.4(8.5–12.3)	126	9.2(7.4–11.6)	0.010	46	11.9(9.7–15.6)	0.006	64	8.1(6.1–10.0)	<0.0001	23	8.3(7.1–10.3)	0.004
Platelets (1000/uL)	117	433 (33–528)	151	406 (314–491)	0.338	67	356 (280–484)	0.043	70	258(186–365)	<0.0001	30	288(177–382)	<0.0001
Serum albumin (g/dL)	113	4.1(3.8–4.4)	124	4.1(3.9–4.3)	0.760	67	4.0(3.7–4.3)	0.145	67	4.0(3.8–4.3)	0.200	29	4.0(3.9–4.5)	0.650
Ferritin (ng/mL)	91	264 (78–772)	152	557 (197–1186)	0.004	55	1990 (472–4478)	<0.0001	50	93(54–180)	<0.0001	22	91(36–282)	0.038

	Severe SCD- No treatment		Severe SCD- On Hydroxyurea			Severe SCD- On Blood Transfusion+			Mild SCD- No Treatment			Mild SCD- On Hydroxyurea		
Marker	**n**	**result**	**n**	**result**	**P***	**n**	**result**	**P***	**n**	**result**	**P***	**n**	**result**	**P***
Cardiopulmonary Function														
Systolic blood pressure (mmHg)	117	119(110–128)	152	119(110.0–127.5)	0.698	66	119(111–128)	0.754	70	120.5(111–130)	0.181	30	125.5(112–131)	0.103
Diastolic blood pressure (mmHg)	117	70(64–74)	152	70(64–76)	0.177	66	72(64–76)	0.125	70	78(70–85)	<0.0001	30	77.5(71–82)	<0.0001
SBP without treatment(mmHg)	102	116(110–125)	117	118(109–126)	0.364	47	113(108–123)	0.297	59	119(110.3–129.0)	0.115	25	124(110–128)	0.157
DBP without treatment(mmHg)	102	70(64–73)	117	70(64–76)	0.103	47	69(63–74)	0.764	59	77(70–81)	<0.0001	25	75.0(70–81)	0.001
NT pro-BNP (pg/mL)	31	53(21–151)	59	47(20–89)	0.619	28	99.5(35.5–285)	0.123	15	23(11–113)	0.241	8	36.5(11.5–55.5)	0.244
NT proBNP >160 pg/mL, n (%)	31	7 (22.6%)	59 8	8 (13.6%)	0.165	28	10 (35.7%)	0.435	15	3 (20%)	0.425	8	1 (12.5%)	0.513
TRV (m/s)	56	2.4(2.2–2.7)	92	2.4(2.2–2.7)	0.758	33	2.5(2.1–2.9)	0.756	24	2.3(2.0–2.6)	0.174	11	2.4(2.1–2.6)	0.379
TRV>2.50 m/sec, n (%)	56	23 (41.1%)	92	42 (45.7%)	0.103	33	15 (45.5%)	0.616	24	7 (29.2%)	0.100	11	3 (27.3%)	0.244
TRV > 3.0 m/sec (%)	56	3 (5.4%)	92	4 (4.3%)	0.944	33	6 (18.2%)	0.050	24	1 (4.2%)	0.626	11	1 (9.1%)	0.795
FEV1 (% Predicted)	24	80.2(69.9–91.3)	42	82.1(73.1–96.1)	0.298	16	64.4 (57.5–95.4)	0.320	11	102.5(92.8–119.7)	0.001	4	88.7(84.9–90.7)	0.341
FVC (% Predicted)	24	86.4(73.9–92.5)	42	85.9(81.3–99.0)	0.172	16	75.4(58.9–103.0)	0.377	11	103.8(93.0–112.5)	0.001	4	91.5(85.1–101.9)	0.189
TLC (% Predicted)	22	83.2–76.8–87.2)	41	83.4(74.2–89.0)	0.746	16	80.4(66.1–86.1)	0.375	9	100(92.9–106.4)	0.001	4	89.7(81.4–96.0)	0.177
DL_{CO} (% ofPredicted)	22	77(69.7–83.6)	41	78.9(60.8–86.5)	0.857	14	66.8(41.5–86.4)	0.330	9	76.4(60.9–96.8)	0.931	4	78.9(60.8–86.5)	0.088
Renal Function														
Serum creatinine (mg/dL)	116	0.7(0.55–0.86)	152	0.66(0.58–0.80)	0.513	67	0.7(0.58–1.03)	0.374	69	0.8(0.7–1.0)	<0.0001	30	0.79(0.68–1.1)	0.012
eGFR*(mL/min/1.73m2)	104	138 (116–149)	136	140 (113–155)	0.317	54	140 (81–158)	0.764	57	108.3(91.4–131.5)	<0.0001	26	124.8(83.0–135.7)	0.003
Urine ACR *(ug/mg)	97	31(12.5–141.9)	130	43.5(14–193.7)	0.307	46	63.8(12–779.7)	0.163	56	10.3(7.0–21.3)	<0.0001	24	6.7(5.3–22.5)	0.001
Hepatic Markers														

	Severe SCD- No treatment		Severe SCD- On Hydroxyurea			Severe SCD- On Blood Transfusion[+]			Mild SCD- No Treatment			Mild SCD- On Hydroxyurea		
Marker	**n**	**result**	**n**	**result**	**P***	**n**	**result**	**P***	**n**	**result**	**P***	**n**	**result**	**P***
Total bilirubin **(mg/dL)	113	2.9(1.8–4.0)	150	2.5(1.7–3.4)	0.105	67	3.0(1.9–4.5)	0.420	67	1.3(0.9–1.7)	<0.0001	29	1.4(0.9–2.1)	<0.0001
Direct bilirubin	112	0.4(0.3–0.6)	140	0.4(0.3–0.6)	0.393	59	0.4(0.3–0.7)	0.398	62	0.2(0.1–0.3)	<0.0001	27	0.2(0.2–0.4)	<0.0001
Aspartate aminotransferase **(U/L)	113	40(28–52.3)	151	36(29.0–45.8)	0.131	67	49(35–61)	0.008	67	25(19.3–30.0)	<0.0001	29	26(19.0–36.5)	0.001
Alanine transaminase (U/L)	113	22(17–29)	151	22(16–31)	0.606	67	30(19–45)	0.001	67	19(13.0–25.5)	0.028	29	21(13.8–27.5)	0.331
Alkaline phosphatase (U/L)	113	83(61.8–110.5)	150	88.5(69–108)	0.256	67	102(79.3–135.0)	<0.0001	66	68.5(56–85)	0.005	29	77(61.3–96.8)	0.384

NT pro-BNP - N-terminal pro b-type natriuretic peptide; TRV- tricuspid regurgitant velocity;

*all p-values were tested against severe SCD not on treatment. P-values <0.002 are significant after the Bonferroni correction.

**These variables can be viewed as hemolytic markers or as hepatic markers.

+25 patients on chronic transfusion were also still receiving hydroxyurea.