Blood Cells, Molecules and Diseases 54 (2015) 321–327

Contents lists available at ScienceDirect

Blood Cells, Molecules and Diseases

journal homepage: www.elsevier.com/locate/bcmd

Severity of Brazilian sickle cell disease patients: Severity scores and feasibility of the Bayesian network model use

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ARTICLE INFO

Article history:
Submitted 16 September 2014
Revised 15 January 2015
Accepted 16 January 2015
Available online 23 February 2015

(Communicated by M. Narla, DSc, 16 January 2015)

Keywords:
Hemoglobin S
Sickle cell anemia
Hydroxyurea

ABSTRACT

The integration of the several clinical and laboratory dimensions and the influence of each parameter on the sickle cell disease (SCD)-related mortality is useful for predicting the phenotype of an individual. This study evaluated the feasibility of the SCD severity calculator use to measure disease severity in Brazilian patients. The study group was composed of 500 SCD patients (440 HbSS and 60 HbSC) diagnosed by molecular biology. We observed a decrease in severity scores in 72 SCD patients assessed before and after the hydroxyurea (HU) use. Furthermore, the HU influenced the increase of mean corpuscular volume (MCV) and HbF concentration, and the decrease of leukocytes and total bilirubin. We found 180 (36.0%) patients with intermediate phenotype, 170 (34.0%) mild phenotype and 150 (30.0%) with severe phenotype. Patients with ages >40 years had higher mean score (0.778 ± 0.177) than patients between 18 and 40 years (0.562 ± 0.152) and patients between 5 and 17 years (0.322 ± 0.145). We observe that there is a tendency of individuals with leg ulcers, avascular necrosis and cardiac complications with increasing age. Correlation analysis showed relations between severity scores with leukocytes, reticulocytes, bilirubin, lactate dehydrogenase, HbS, hemoglobin and hematocrit ($p < 0.05$). Several comparisons involving age groups, SCD genotype and phenotypic classification had satisfactory results and this classification will be used for future studies involving genetic polymorphisms, response to treatment with HU and oxidative stress markers in SCD.

1. Introduction

Sickle cell disease (SCD) is characterized by a very heterogeneous clinical course between patients with the same mutations for sickle hemoglobin (HbS), ranging from patients who have normal life expectancy with relatively few complications. Others can have severe complications such as pulmonary hypertension, priapism, stroke, leg ulceration, recurrent painful episodes, acute chest syndrome (ACS) and avascular necrosis of bone (AVN) [1].

The molecular basis for HbS formation is known, but only the mutation (HBB, glu6val, rs334) is not sufficient to explain the heterogeneous phenotype found in SCD patients, other factors such as HbF levels [2–7], α-thalassemia co-inheritance [8–13], genetic polymorphisms [14–21], hydroxyurea use [22–26] and environmental factors [27] have been identified as modulators of SCD.

Even with improved knowledge of the human genome, development of new genomic tools and identification of single nucleotide polymorphisms (SNPs) associated with sub-phenotypes of SCD by genome-wide association studies (GWAS) [28], there is still a major challenge to combine all these variables and establish potential predictors of the SCD severity. Thus, the knowledge of these determinants may help to unravel the pathophysiological mechanisms underlying the disease process, to identify novel targets for therapeutic interventions, and to enhance patient care [29].

On the other hand, a number of SCD severity classifications have been proposed, aiming at the integration of many clinical and laboratory dimensions and the influence of each parameter on disease-related mortality [29–33]. Therefore, a meaningful single synthetic measure of morbidity and/or risk of death within a given period is clinically useful to understand the relationships among clinical and laboratory measures of disease expression and to identify genetic variants that impact the disease severity [34].

An impediment to this objective has been the inability to integrate the many clinical and laboratory dimensions of the disease into a single measure of disease severity using traditional statistical methods. Furthermore, many results of hematological and biochemical tests are

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http://dx.doi.org/10.1016/j.bcmd.2015.01.011
1079-9796/

usually found outside of normal values hindering the prediction of disease severity.

Sebastiani et al. [35] developed a predictive model of disease severity using a Bayesian network modeling approach in which the 5-year mortality risk was projected as a disease severity score and can be used in other studies involving clinical and laboratory variables. However, the data on the patients were derived from United States residents and the application of this tool in individuals from others populations like in Brazil should be tested.

In this study, we aimed to evaluate the feasibility of the calculator use for disease severity (http://www.bu.edu/sicklecell/downloads/Projects) and to classify the Brazilian SCD patients according to the range of scores established.

2. Methods

2.1. Study design, patients and data collection

An observational study involves 500 SCD patients (237 males and 279 females with a mean age of 25.1 ranging between 5 and 65 years old) receiving medical care at State Institute of Hematology "Arthur de Siqueira Cavalcanti", Rio de Janeiro (HEMORIO). Eligible patients were 5 years old or older at the beginning of the study. Patients were excluded if they were smokers, consumers of alcoholic drinks, pregnant, or if they had a stroke, pain, or a hemolytic crisis in the 4 weeks prior to the date of collection of blood sample for routine laboratory tests. From this date of collection made a retrospective assessment to obtain the clinical events, hydroxyurea (HU) treatment and regular blood transfusions for the severity score calculation. We considered the patients who were using hydroxyurea over 90 days. All patients were under follow-up according to Clinical Protocols and Therapeutic Guidelines for SCD patients of Brazil [36,37].

A group of 72 SCD patients (mean age of 36.4 years old) randomized and adherent to HU treatment from HEMORIO, were studied to assess the HU effect on the severity scores. The severity score was calculated in patients without the HU use and after a mean of 105 days with HU use, the scores were recalculated. The eligible and exclusion criteria, as well as, therapeutic guidelines, followed the same principles mentioned previously.

2.2. Hemoglobin phenotypes and genotypes

Hemoglobin was identified using electrophoresis on cellulose acetate at pH 8.6, and using agar electrophoresis at pH 6.2. Hb fraction quantification was obtained using high performance liquid chromatography (HPLC) with the automated VARIANT™ equipment (Bio-Rad Laboratories, CA, USA). In all samples, the Hb genotype was developed through molecular analysis using PCR–RFLP and PCR–EA [38].

2.3. Sickle cell disease severity calculator

We used the tool "Sickle Cell Disease Severity Calculator", available from http://www.bu.edu/sicklecell/downloads/Projects, for calculation of severity scores and classification of patients into categories by phenotype (mild, intermediate, severe). This tool was developed through a Bayesian network modeling using 25 clinical and laboratory variables to estimate the severity of SCD in a study involving 3380 patients followed in the Cooperative Study of Sickle Cell Disease (CSSCD). The network model calculates the death risk within 5 years and considers this risk as a disease severity score, which ranges from 0 (least severe) to 1 (most severe). The predictive value (ie, accuracy of forecasting death based on a clinical and laboratory profile) of the model was validated in two unrelated sets of patients and showed high specificity and sensitivity [35].

The calculator requires the following variables that are sufficient to compute the score for severity of disease: age, acute chest syndrome (ACS), serum total bilirubin, blood transfusion, lactate dehydrogenase (LDH), mean corpuscular volume (MCV), pain crises, priapism, reticulocyte count, gender, stroke, total leukocyte (WBC), genotype of SCD and avascular necrosis of bone (AVN). Bilirubin, LDH, MCV, reticulocyte, and WBC levels were obtained from the collection date of samples for routine laboratory tests. Blood transfusion was assigned to patients who have regular blood transfusion for more than 1 year before the start of the study. Priapism and ACS were considered as variables present in patients with these frequent events near collection date of blood samples. Painful crises were considered present in patients who had more than 3 episode crises per person per year in the last year before the date of sample collection.

Two parameters required by the calculator "Sepsis" and "systolic blood pressure" could not be used for this study because they were not available in the routine medical records, in Brazil. Even in the absence of any variable (clinical or laboratory) the calculator allows the researcher to enter "non available" for the missing variable. Thus, the calculator fixes a reference value and the changes in the risk for death changes according to the other variables filled.

2.4. Statistical analysis

Statistical analysis was performed using the Statistica 11.0 and Graphpad Prism 5.0 softwares. Data are expressed as mean ± standard deviation and were tested for normal distribution using the Lilliefors test. Data were also tested for homogeneity of variances using Levene's test. Means between the groups were compared by applying the t-test or one-way ANOVA, followed by the post hoc Tukey's test for parametric data, and either the Mann–Whitney test or Kruskal–Wallis test, followed by the post hoc Dunn's test for non-parametric data. To assess association degree between the studied variables, we performed Pearson's correlation for parametric data and Spearman's correlation for non-parametric data. Categorical data were compared by Binomial test for proportion analysis, and Pearson Chi-square test supplemented by Fisher's exact test, for association analysis. A p value of <0.05 was considered statistically significant.

3. Results and discussion

This is the first Brazilian study involving SCD patients that assesses the feasibility of "sickle cell disease severity calculator" to calculate the severity scores of disease and classify patients into phenotypic categories. In this regard, we conducted several comparisons involving age groups, SCD genotype, HU use and phenotype classification.

We separated the patients into three age groups according to calculator (age below 18 years, age between 18 and 40 years, age above 40 years) and evaluated the HU influences on the disease severity, hematological and biochemical data between patients using and not using HU (Table 1). The HU influenced in the increase in mean corpuscular volume (MCV) and HbF concentration. Furthermore, we observed a decrease of leukocytes amount and of total bilirubin levels in the HU-sample groups. The beneficial effects of HU as increased HbF synthesis, increased hemoglobin and VCM, and decreased hemolysis markers and number of leukocytes have been characterized in the literature [25,39–41].

Severity scores in three age groups were not statistically different between patients using and not using HU. This difference was not found because the calculation of the scores was a punctual assessment and we do not calculate the baseline score severity in patients using HU. In this sense, when we evaluated 72 patients before and after the use of HU, we checked the HU influence on various laboratory and clinical data, as shown in Table 2. As expected, we observed an increased HbF synthesis, increased Hb, MCV and hematocrit values, furthermore, decreased of leukocyte number, and decreased reticulocyte and bilirubin levels.

Table 1
Demographics and clinical data and laboratory values in SCD patients separated into age groups with and without hydroxyurea use.

Characteristics	5–17 years		p value	18–40 years		p value	>40 years		p value
	−HU (n = 108)	+HU (n = 82)		−HU (n = 143)	+HU (n = 79)		−HU (n = 43)	+HU (n = 45)	
Hb genotypes (HbSS/HbSC)	89/19	79/03	–	125/17	74/5	–	34/09	39/06	–
Gender (female/male)	48/60	34/48	–	87/56	42/37	–	33/10	29/16	–
Hemoglobin (g/dL)	9.5 ± 6.4	8.6 ± 1.4	0.15	8.8 ± 1.8	9.0 ± 1.7	0.24	8.5 ± 1.9	8.7 ± 1.9	0.7
Hematocrit (%)	25.9 ± 7.8	24.7 ± 4.4	0.38	25.1 ± 5.5	25.7 ± 5.1	0.31	24.5 ± 6.2	24.4 ± 5.8	0.95
MCV (fL)	84.1 ± 9.0	94.0 ± 9.9	<0.001	87.8 ± 8.2	97.4 ± 12.2	<0.001	87.6 ± 9.1	98.1 ± 22.9	<0.001
Leucocytes (/mm^3)	10.7 ± 3.3	9.9 ± 3.2	0.04	10.8 ± 3.4	9.9 ± 5.0	<0.001	10.9 ± 4.4	8.2 ± 3.4	0.03
Reticulocyte (k/uL)	248.2 ± 112.5	240.2 ± 91.4	0.36	252.7 ± 99.9	229.0 ± 93.8	0.09	200.3 ± 102.9	177.2 ± 79.2	0.32
LDH (U/L)	1081.9 ± 567.3	953.0 ± 343.5	0.12	940.3 ± 482.6	873.3 ± 416.5	0.31	927.7 ± 519.1	750.1 ± 79.2	0.12
Total bilirubin (mg/dL)	3.4 ± 2.2	2.7 ± 1.9	0.01	3.7 ± 2.6	2.8 ± 2.2	0.01	3.1 ± 2.2	2.2 ± 1.4	0.03
ALT (U/L)	23.1 ± 15.3	22.6 ± 13.5	0.58	30.4 ± 29.6	33.6 ± 25.8	0.93	25.2 ± 14.3	26.1 ± 16.3	0.98
AST (U/L)	54.1 ± 22.1	52.9 ± 22.1	0.66	54.7 ± 28.4	56.9 ± 46.0	0.47	50.6 ± 21.2	52.9 ± 30.1	0.65
Creatinine level (mg/dL)	0.6 ± 0.1	0.6 ± 0.1	0.92	0.7 ± 0.2	0.8 ± 0.2	0.12	1.2 ± 1.1	1.1 ± 0.5	0.18
HbF (%)	5.6 ± 4.8	9.9 ± 5.5	<0.001	4.8 ± 4.6	8.8 ± 7.1	<0.001	5.7 ± 4.8	11.7 ± 9.3	<0.001
Blood transfusion [n (%)]	39 (36.1%)	45 (54.9%)	0.009	53 (37.1%)	47 (59.5%)	0.013	14 (32.5%)	21 (47.0%)	0.17
Number of sickle cell crises per person per year in the last year [n (%)]									
0–2	81 (75.0%)	50 (61.0%)	0.07	101 (70.6%)	33 (41.8)	<0.001	25 (58.1%)	29 (64.4%)	0.38
3–5	24 (22.2%)	22 (26.8%)	0.62	29 (20.3%)	30 (38.0%)	<0.001	13 (30.2%)	11 (24.4%)	0.63
≥6	03 (2.8%)	10 (12.2%)	0.01	13 (9.1%)	16 (20.2%)	0.02	05 (11.6%)	05 (11.1%)	0.52
Complications of sickle cell disease [n (%)]									
Stroke	22 (20.4%)	16 (19.5%)	0.71	22 (15.4%)	12 (15.2%)	0.92	07 (16.3%)	04 (8.9%)	0.29
Leg ulcers	04 (3.7%)	01 (1.2%)	0.26	30 (21.0%)	22 (27.8%)	0.28	19 (44.2%)	25 (55.5%)	0.22
Avascular necrosis	02 (1.9%)	01 (1.2%)	0.68	11 (7.7%)	08 (10.1%)	0.56	12 (27.3%)	11 (24.4%)	0.83
Acute chest syndrome	47 (43.5%)	58 (70.3%)	<0.001	81 (56.6%)	61 (77.2%)	<0.001	18 (41.0%)	21 (46.7%)	0.51
Cardiac complications	02 (1.9%)	03 (3.6%)	0.48	15 (10.5%)	10 (12.6%)	0.66	12 (27.3%)	12 (27.1%)	0.99
Priapism	04 (7.0%)	10 (20.8%)	0.03	12 (21.4%)	14 (37.8%)	0.04	02 (20.0%)	03 (18.7%)	0.64
Mean severity score	0.320 ± 0.147	0.324 ± 0.144	0.85	0.549 ± 0.145	0.585 ± 0.164	0.09	0.795 ± 0.194	0.762 ± 0.161	0.38

MCV (mean corpuscular volume); LDH (lactate dehydrogenase); ALT (alanine aminotransferase); AST (aspartate aminotransferase); −HU (patients without hydroxyurea use); +HU (patients hydroxyurea use). Cardiac complication (congestive heart failure, cardiomegaly, cardiomyopathy and myocardial infarction).
We considered the patients who were using hydroxyurea over 90 days. Quantitative data comparisons were made by Mann–Whitney test (non-parametric data) and t-test (parametric data). Categorical data comparisons were made by Binomial test.

Significant decrease in severity scores ($p = 0.02$) in patients using HU was possible because there was an improvement of laboratory indices, as well as in the reduction of patients with painful crises and other clinical events (even not statistically significant). The small decrease in scores on the HU influence may be due to association strength in that each variable represents in the network [35]. For example, the variables painful crises and MCV has a low strength of association (odds ratio (OR) of 1.98 and 1.61, respectively) when compared with the stroke that has a higher OR of 3.8. Precisely this variable (stroke) may have influenced the overall mean scores of patients using HU because there was an increase of three patients in this group.

Table 2
Clinical data and laboratory values in SCD patients before and after the hydroxyurea use.

Characteristics	Before HU use	After HU use	P value
Hemoglobin (g/dL)	8.2 ± 1.8	8.8 ± 1.7	0.0017
Hematocrit (%)	23.4 ± 5.6	24.9 ± 5.2	0.0023
MCV (fL)	91.2 ± 13.7	99.6 ± 17.7	0.0008
HbF (%)	6.5 ± 5.6	12.2 ± 7.7	<0.0001
Leucocytes (/mm^3)	10.7 ± 4.0	9.1 ± 4.6	0.0088
Reticulocyte count (%)	12.2 ± 5.2	8.4 ± 4.1	<0.0001
LDH (U/L)	768.2 ± 505.6	746.5 ± 335.3	0.6909
Total bilirubin (mg/dL)	3.5 ± 2.6	2.6 ± 1.9	<0.0001
Complications of sickle cell disease [n (%)]			
Blood transfusion	32 (44.4%)	27 (37.5%)	0.3969
Painful crises (more than 3 episodes in last year)	25 (34.7%)	13 (18.1%)	0.0233
Stroke	7 (9.7%)	10 (13.9%)	0.4385
Priapism	12 (44.4%)	10 (37.0%)	0.6432
Mean severity score	0.641 ± 0.194	0.601 ± 0.223	0.0281
Classes of phenotype [n (%)]			
Mild	07 (9.7%)	16 (20.8%)	0.0406
Intermediate	30 (41.7%)	31 (43.1%)	0.7364
Severe	35 (48.6%)	25 (34.7%)	0.0910

MCV (mean corpuscular volume); LDH (lactate dehydrogenase); ALT (alanine aminotransferase); AST (aspartate aminotransferase). Quantitative data comparisons were made by paired t-test. Categorical data comparisons were made by Binomial test.

According to the severity scores distribution, in the age group between 5 and 17 years, 13.2% of patients had scores greater than 0.5; the age group between 18–40 years, 60.8% of patients were above 0.5 and in the age group >40 years, 90.8% of the patients had risk of death above 0.5 (Fig. 1). Furthermore, independent of HU use, patients age >40 years had the highest mean score (0.778 ± 0.177) followed by patients of age between 18 and 40 years (0.562 ± 0.152) and finally patients age between 5 and 17 years (0.322 ± 0.145) ($p < 0.0001$). These observations demonstrate that age is a factor that influences the disease severity and the calculator was accurate for the age, i.e., the risk of death increases with age and this observation has been well documented once age is related to the natural history of SCD [42–44].

The frequency of phenotypes among the age groups presented statistical difference. We observed that there is a tendency of higher number of individuals with leg ulcers, avascular necrosis and cardiac complications with increasing age. The occurrence of priapism in men and number of painful crisis were lower in subjects between 5 and 17 years compared with other age groups (Table 3). We believe that this gravity might be a consequence of two factors in older patients: one would be the management and diagnosis of the disease that were still in improvement process in Brazil in previous decades, and the other factor would be the less knowledge of the pathophysiology and treatment of the disease which had at that time [45].

Regarding to the genotype of the disease, the mean severity scores were higher in HbSS (0.501 ± 0.218) compared with HbSC (0.395 ± 0.223), ($p < 0.001$). Patients with HbSC disease are usually less severe than individuals with sickle cell anemia and HbS-β^0 thalassemia [46]. On average, individuals with HbSC disease have half the number of painful episodes than sickle cell anemia patients. Stroke is less frequent

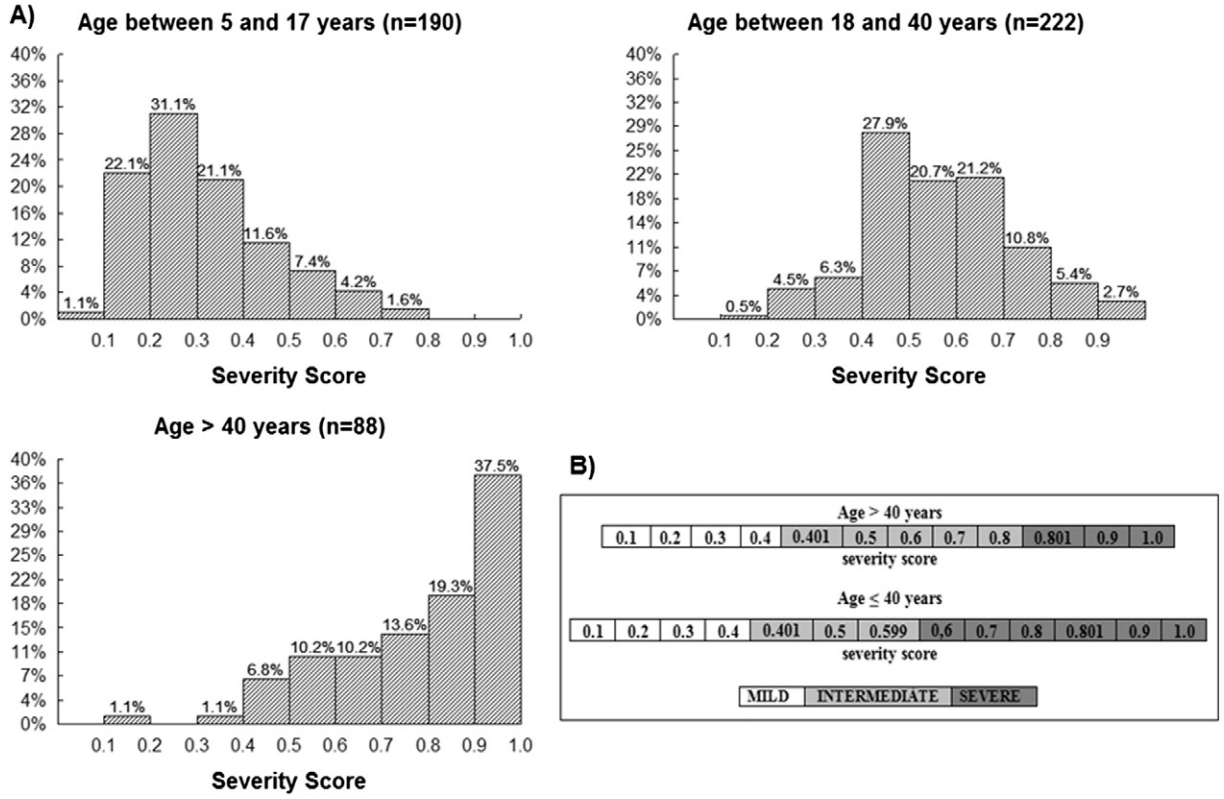

Fig. 1. Distribution of the severity score in sickle cell disease patients and score ranges for phenotypes classification. (A) The histograms show the different distribution of disease severity score in three age groups. In ascending order, the frequency of patients with more severe scores: [age > 40 years] > [age between 18 and 40 years] > [age between 5 and 17 years]. (B) Scores ranges for phenotype classification in mid, intermediate and severe.

and leg ulcers are rare in HbSC patients [30,47,48]. Thus, the calculator showed a sensitivity of severity in the aspect of the SCD genotypes.

In order to classify patients into phenotypes classes (mild, intermediate and severe), we used the score ranges established in the work Sebastiani et al. [49], and adapted for our study. The classification was based on the observation that the severity score has a U shape that changes in age groups. Therefore, the scores ≤0.4 were classified as "mild phenotype" independent of age. The score >0.8 for patients age >40 years and scores ≥0.6 for patients age ≤40 years were considered as "severe phenotype". For patients age >40 years, the score >0.8 was considered for severe phenotype to reduce the risk of misclassification. The scores that do not correspond to "mild" and "severe" phenotype were classified as "intermediate" phenotype (Fig. 1B). After determining the score ranges, we classified the SCD phenotypes and we found that 180 (36.0%) patients had intermediate phenotype, 170 (34.0%) mild phenotype and 150 (30.0%) the severe phenotype.

For the use of phenotypic classification in future analyses, we verified whether the SCD complications were associated with the phenotype class. In Table 4, we found the association of severe phenotype with stroke, leg ulcers, cardiac complications and blood transfusion. Pain crises of 3–5 per person in the last year and avascular necrosis were more frequent in intermediate and severe phenotypes when compared with the mild phenotype. The SCD severity is based on the number of painful episodes, frequency of hospital admissions, priapism, history of stroke, acute chest syndrome or sepsis, chronic leg ulcers, pulmonary hypertension, renal impairment, and avascular necrosis of

Table 3
Complications of sickle cell disease separated according to age groups.

Complications of SCD	Age groups			P value
	5–17 years (n = 190)	18–40 years (n = 222)	>40 years (n = 88)	
Stroke	38 (20.0%)	34 (15.3%)	11 (13.2%)	0.23
Leg ulcers	5 (2.6%)[a]	52 (23.4%)[b]	44 (50.0%)[c]	<0.001
Avascular necrosis	3 (1.6%)[a]	19 (8.6%)[b]	23 (26.5%)[c]	<0.001
Acute chest syndrome	106 (55.8%)	142 (63.9%)	39 (44.3%)	0.107
Cardiac complications	5 (2.6%)[a]	25 (11.3%)[b]	24 (27.3%)[c]	<0.001
Priapism [n/total male]	14/108 (12.9%)[a]	26/93 (27.9%)[b]	5/26 (19.2%)[b]	0.025
Blood transfusion	84 (44.2%)	100 (45.1%)	35 (39.8%)	0.065
*Painful crises [0–2]	82 (43.2%)[a]	134 (60.4%)[b]	54 (61.4%)[b]	<0.001
*Painful crises [3–5]	24 (12.6%)[a]	59 (26.6%)[b]	24 (27.3%)[b]	<0.001
*Painful crises [≥6]	7 (3.7%)[a]	29 (13.1)[b]	10 (11.4%)[b]	0.003

Data were expressed as number of patients with complication (percent of patients). Different letters indicate statistical difference (Pearson Chi-square test supplemented by Fisher's exact test).
* Number of sickle cell crises per person per year in the last year.

Table 4
Complications of sickle cell disease separated according to phenotypes characterized by ranges of severity scores in mild, intermediate and severe.

Complications of SCD	Phenotypes			P value
	Mild (n = 170)	Inter (n = 180)	Severe (n = 150)	
Stroke	21 (12.4%)[a]	25 (13.9%)[a]	37 (24.7%)[b]	<0.01
Leg ulcers	9 (5.3%)[a]	43 (23.9%)[b]	49 (32.7%)[c]	<0.001
Avascular necrosis	4 (2.3%)[a]	23 (12.8%)[b]	18 (12.0%)[b]	0.002
Acute chest syndrome	97 (57.1%)	94 (52.2%)	95 (63.3%)	0.067
Cardiac complications	6 (3.5%)[a]	21 (11.7%)[b]	27 (18.0%)[c]	0.006
Priapism [n/total male]	11/88 (12.5%)	23/83 (27.7%)	11/54 (20.4%)	0.081
Blood transfusion	59 (34.7%)[a]	60 (33.3%)[a]	100 (66.7%) [b]	<0.001
*Painful crises [0–2]	117 (68.8%)	119 (66.1%)	83 (55.3%)	0.241
*Painful crises [3–5]	40 (23.5%)[a]	48 (26.7%)[b]	41 (27.3%)[b]	0.001
*Painful crises [≥6]	13 (7.7%)[a]	13 (7.2)[a]	26 (17.3%)[b]	0.122

Data were expressed as number of patients with complication (percent of patients). Different letters indicate statistical difference (Pearson Chi-square test supplemented by Fisher's exact test).
* Number of sickle cell crises per person per year in the last year.

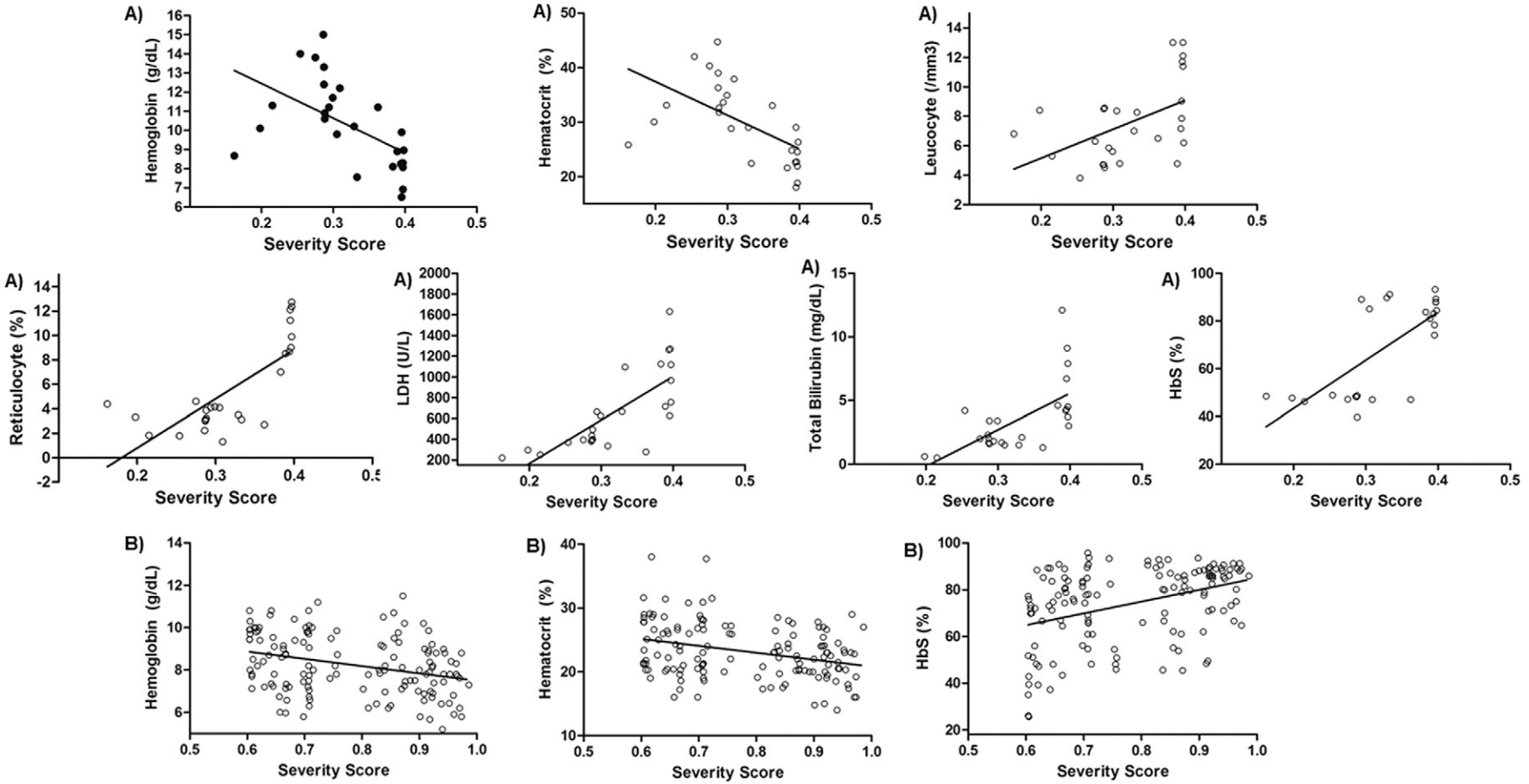

Fig. 2. Correlation analyzes using severity scores and laboratory variables. A) Correlation analysis in mild phenotype without the group 5–17 years. The degree of relation between severity score (ssco) and hemoglobin ($p < 0.001$, $r = -0.65$) and ssco and hematocrit ($p < 0.001$, $r = -0.68$) were significantly negative. The degree of relation between ssco and leucocyte ($p < 0.001$ and $r = 0.54$), ssco and reticulocyte ($p < 0.01$ and $r = 0.64$), ssco and LDH ($p < 0.001$ and $r = 0.76$) ssco and bilirubin ($p < 0.01$ and $r = -.31$) and ssco and hematocrit ($p = <0.01$ and $r = -0.32$) were significantly negative. The degree or relation between ssco and HbS ($p < 0.001$ and $r = 0.34$) was significantly positive. When hemoglobin and hematocrit values decrease the severity score values tended to increase; and when leucocyte, reticulocyte, LHD, bilirubin and HbS values increased the severity score values tended to increase.

bones [50,51]. Therefore, we demonstrated that the disease manifestations were associated with the classes of severe phenotypes.

The few studies associated with disease severity and severity scores have shown lower scores than those found in Brazilian patients. Anoop et al. [52] found in patients >18 years classified phenotypically by clinicians, mean score of 0.182 for the mild phenotype, score of 0.19 for the intermediate and mean score of 0.866 for the severe phenotype. However, these patients also had lower frequencies of individuals with priapism (18%), ACS (14%) and stroke (12%) compared to Brazilian patients >18 years that showed 26% of men with priapism, 58% of patients with ACS and 31% stroke patients. Patients >21 years from Boston Medical Centre showed the following mean severity scores: mild phenotype (0.16), intermediate phenotype (0.28) and severe (0.95) [35]. These results show that regardless of the form of phenotypes categorization, Brazilian patients have higher mean scores, as shown: mild phenotype (0.32), intermediate phenotype (0.54) and severe (0.80).

We evaluated in all patients the relationship of biomarkers with severity scores and the degree of relation between severity score (ssco) and hemoglobin ($p = 0.004$, $r = -0.20$), ssco and hematocrit ($p = 0.001$, $r = -0.21$), ssco and leukocyte ($p = 0.02$, $r = 0.10$), ssco and reticulocyte ($p = 0.009$, $r = 0.11$) were statistically significant. Since there is no validation of severity calculator in patients <18 years [35], we remove the group of 5–17 years and have made other correlation analyses. We found the relation between ssco and hemoglobin ($p < 0.001$, $r = -0.32$), ssco and hematocrit ($p < 0.001$, $r = -0.33$), ssco and leukocyte ($p = 0.03$, $r = 0.26$), ssco and reticulocyte ($p < 0.001$, $r = 0.27$), and ssco and LDH ($p < 0.001$, $r = 0.28$). These results show that when LDH, leucocyte and reticulocyte values increased, the ssco values tended to increase. On the other hand, when hemoglobin and hematocrit values decreased the ssco values tended to increase. In addition, we analyzed the degree correlation between severity scores and laboratory variables within each phenotype class (without the patients aged 5–17 years) and the results are shown in Fig. 2.

The correlation analysis allowed us to verify that the disease severity is associated with decreased hemoglobin and hematocrit. Low hemoglobin is linked generally to poor prognosis in SCD with increased risk of many specific complications [30,53–55]. In the first analysis, the correlation coefficients were not high, but when we removed the group 5–17 years, the correlation coefficients increased and other variables became significant. Furthermore, we found that the SCD severity is associated with increased of leukocytes, reticulocytes, bilirubin, LDH and HbS. All these markers are associated with the severity of the disease, e.g., increased leucocytes have been correlated with increased frequency of pain [51], increased hemorrhagic stroke risk [6] and earlier death [2]; increased LDH, bilirubin and reticulocytes are laboratory indicators of chronic hemolytic anemia [48,56,57], and the increase in HbS concentration provides the polymerization, under specific conditions [58,59].

Currently there are two models of disease severity that have been validated by other studies: one involving pediatric patients [29] and another which we are validating [35]. Thus discussion among different studies involving severity tools is limited, Coelho et al. [34] mentioned that both tools are not yet the effective tools needed for patient stratification in genotype/phenotype relationship analysis as well as in the discovery and validation of prognosis markers of the largely unpredictable SCD clinical course. Anoop et al. [52] agree that scores have high specificity and positive predictive value, but for patients >40 years start off with a high score and if data on one or more severity parameters for such patients are unavailable, the score is likely to be spuriously high.

Importantly, there are already results in the literature showing the relationship between genotype and severity score. Studies have reported the association of several SNPs (for example, rs652785 in complement component 8 gene-C8A) and some biomarkers (tumor necrosis factor-α receptor-1 and vascular cell adhesion molecule-1) with the severe phenotype classified by severity calculator [49,60]. Thereby, demonstrating the applicability of the calculator for patient clinic association with genetic and biochemical markers.

The correlation analysis and comparisons of clinical manifestations in different groups helped to evaluate the feasibility of tool. Furthermore, the group of patients <18 years deserves more attention, because the calculator does not contain clinical and laboratory variables specific to this age which may compromise the generation severity score. Another observation was in patients classified as intermediate phenotype. In this group, we have not found significant correlations as in the other two phenotypes classes (mild and severe). In this group, we found patients with very heterogeneous hematological and biochemical characteristics, which prevented the significant correlations. Maybe a subdivision of the intermediate phenotype could be done in these patients, fulfilling other criteria for classification. Moreover, the incorporation of genetic polymorphisms that participate in relevant pathological events of SCD (e.g., rapid destruction of sickle cells, dense cell formation, and adhesion to endothelium) might improve the utility of the scoring system.

For our study the severity calculator, showed high sensitivity and positive predictive value. Several comparisons involving age groups, SCD genotype, HU use and phenotype classification had satisfactory results and this classification will be used in future studies involving genetic polymorphisms, response to treatment with hydroxyurea and oxidative stress markers in SCD.

Authorship

E.B.J.: data design, data acquisition, data analysis, data interpretation, and manuscript preparation. D.G.H.S.: data interpretation and statistical analysis assistance. L.S.T.: technical and statistical analysis assistance. J.V.O.: technical and statistical analysis assistance. C.L.C.L.: study concept and design and critical review of manuscript. C.R.B.D.: study concept and design and critical review of manuscript.

Acknowledgments

The authors would like to thank the following Brazilian foundations: Conselho Nacional de Desenvolvimento Científico e Tecnológico — CNPq (grant 409691/2006-2), Ministerio da Saude (grant MS 3072/2007), and Fundaçao Pro HEMORIO — Fundarj (grant 188/13) for their financial support.

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