SICKLE CELL DISEASE SEVERITY SCORING: CROSS-VALIDATION BETWEEN A DISEASE SEVERITY SCORE AND A PAEDIATRIC SEVERITY SCORE

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(In www.abbotlab.wordpress.com)
Fig.1. Normal red blood cells and a sickled one.

Introduction

Sickle Cell Disease (SCD), one of the most common autosomal recessive hereditary anemia, is caused by a mutation in the β-globin gene (HBB:c.20A>T) on 11p15.5. This originates a hemoglobin variant named HbS, as opposed to the normal adult HbA. HbS ability to polymerize when deoxygenated gives rise to abnormal sickled red blood cells (Fig.1).

SCD is a paradigm of a monogenic disorder under polygenic and environmental control. This aetiopathogenic architecture leads to marked clinical heterogeneity with the emergence of multiple and diverse subphenotypes, which makes the patients severity stratification particularly difficult[1].

A number of severity scores have been proposed, aiming at the integration of many clinical dimensions into a meaningful single synthetic measure of morbidity and/or risk of death within a given period.

As part of a wider research on the development and validation of vaso-occlusion early predictors in SCD, we have analysed (i) the correlation between two thoroughly developed scores, namely a disease severity score (DSS)[2] and a paediatric severity score (PSS)[3], and (ii) the association of scores to a number of genotypic and phenotypic markers in a series of 99 paediatric SS patients.

Results

➢ The evaluated patients were mostly (97%) of Sub-Saharan African ancestry and presented an M/F ratio of 1.17, a median current age of 9.9 years, and a median follow-up/patient of 5.0 years (Table I).

Table I. Characterisation of SCD (SS) patient population sample

No. of patients (sex; ethnicity)	**99** (54% males; 61% Angolan; 97% of Sub-Saharan origin)
Entry age (year)	
median	2.3
interquartile range	0.7-4.7
total range	0.1 – 16.9
Current age[a] (year)	
median	9.9
interquartile range	6.7-12.6
total range	2.9 – 21.7
Total follow-up (person*year)	557
Follow-up/patient (median; year)	5.0
No. of contributing clinical centres	2 Paediatric Departments

[a] At the end of data collection

➢ Although statistically significant, this weak positive correlation, coupled with a fair inter-rater agreement (κ value) of 0.281, suggests **the compared severity scores, although overall convergent, are measuring different aspects of the phenotype, at different developmental stages (paediatric versus adult) and with different weights (Fig. 2).**

➢ Both DSS and PSS displayed a non-normal ($p<0.01$) multimodal distribution. The Spearman's ρ correlation coefficient between DSS and PSS was 0.280 which is significant at the 2-tailed 0.01 level.

Regarding the **association studies**, statistically significant relationships are observed , after correction for the false discovery rate:

➢ **DSS and PSS are both negatively associated with high fetal hemoglobin (Hb F) levels (Fig. 3.A, 3.B and 3.C). HbF level was the only biomarker that strongly associates with both severity scores.**

➢ **DSS** is positively associated with a beta-globin gene cluster polymorphism (data not shown), leukocyte count and RDW (**Fig 3D and 3.E**).

Conclusions

➢ Recently developed SCD severity scores are not yet the effective tool needed for patient stratification in genotype/phenotype (including response to medical interventions) association studies, as well as in the discovery and validation of prognosis markers of the largely unpredictable SCD clinical course. In particular, the marked reduction of sepsis incidence in younger SCD patients in developed countries is just an illustration of the type of problems novel improved pediatric severity scores should address.

➢ Concerning HbF, our results support previously data. In fact, HbF expression is the major determinant of phenotypic severity in SCD. It is the most important modulator of the clinical and hematological features of the disease because it is unable to enter the HbS polymer and reduces mean corpuscular HbS concentration.

➢Disease severity association with high leucocyte count may reflect chronic inflammation, whereas its association with elevated RDW denotes anisocytosis and, possibly, an increased number of irreversible sickle cells.

References

1. Steinberg MH. Predicting clinical severity in sickle cell anaemia. Br J Haematol 2005, 129:465.
2. Sebastiani P *et al.* A network model to predict the risk of death in sickle cell disease. Blood 2007, 110:2727.
3. Van den Tweel XW *et al.* Development and validation of a pediatric severity index for severity cell patients. Am J Hematol 2010, 85:746.
4. Steinberg MH and Sebastiani P. Genetic modifiers of sickle cell disease. Am J Hematol 2012, 87:795.

Acknowledgements

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Methods

Subjects: 99 paediatric SS patients (Table I) followed-up in two general hospitals in Greater Lisbon area.
Candidate gene genotyping: *BCL11A, CD36, EDN1, HBA, HBB* cluster (including *HBG)*, *HBS1L-MYB*, *ITGA4, HMOX1, NOS3*, *THBS1* and *VCAM1.*
Statistical analysis: Data were extracted from hospital records and entered into a database. The normality of scores distribution was tested with Shapiro-Wilk test (α=0.05) and their statistical relationship was evaluated by Spearman correlation. Inter-rater agreement regarding the severity groups defined by each score was assessed by weighted kappa measure.
Association studies between severity scores and candidate genotypes, as well as with hematological and biochemical biomarkers were performed using T/test ANOVA parametric tests or Mann-Whitney/Kuskal-Wallis non-parametric tests, using the false discovery rate (FDR) for multiple testing correction.
All the statistical analysis was performed with SPSS v20.

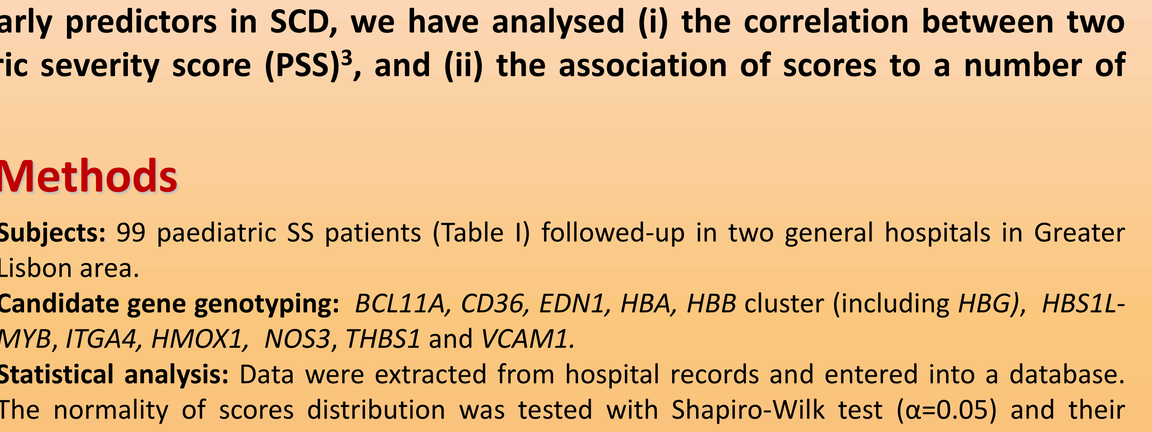
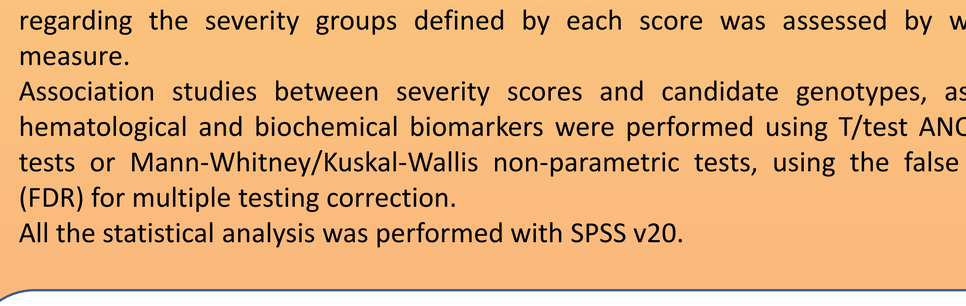
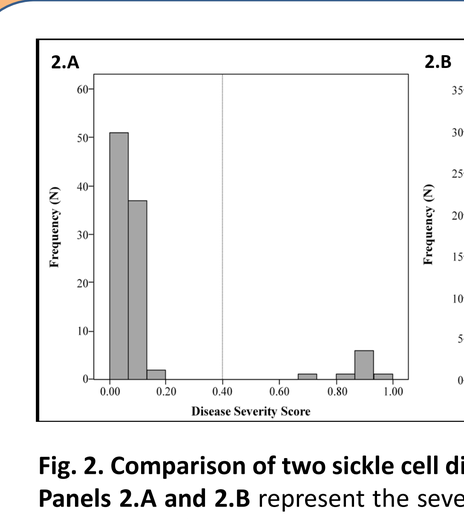


Fig. 2. Comparison of two sickle cell disease severy scores.
Panels 2.A and 2.B represent the severity distribution of 99 SS subjects according to Disease Severity Score or Paediatric Severity Score, respectively. Vertical lines represent the border between lower and higher clinical severity. Severity score cut-off values were arbitrary set as DSS = 0.400 and PSS = 40.
Panel 2.C depicts a scatter plot correlating the two scores in the same group of patients.
Note that 20 out of the 99 patients scored discordantly by DSS and PSS criteria.





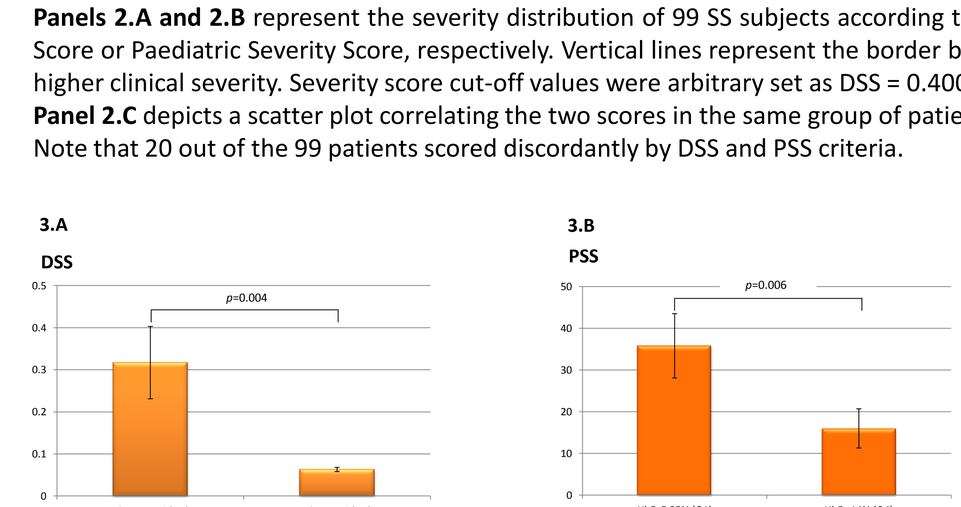


Fig. 3. Statistically significant associations between severity scores and serum hematological parameters.
Panels 3.A and 3.B show lower severity scores (concerning both DSS and PSS) when high levels of HbF are present (upper quartile).
Panel 3.C reinforces the above result – lower severity scores (using DSS categorical data) are preferentially found associated with higher HbF levels.
Panel 3.D and 3.E reveal a positive association between DSS and leucocyte count as well as RDW.