Chapter 13
Hemoglobin Sβ Thalassemia, SC Disease and SD Disease: Clinical and Laboratorial Aspects

Sara T. Olalla Saad and Simone O. Gilli

Abstract Sickle cell disorders are inherited hemolytic anemias, associated with the presence of Hemoglobin S. This group of disorders comprises homozygotes (HbSS), compound heterozygotes for hemoglobin C (HbSC) or β-thalassemia (Sβ thalassemia) (the most frequent associations) and, uncommonly, hemoglobin D (HbSD) and hemoglobin E (HbSE). This abnormal phenotype is caused by mutations in the Beta globin genes of both chromosomes 11. Thus, these disorders are recessively inherited and abnormalities in both alleles lead to structural defects in the beta-globin chain (HbS, HbC, HbD, HbE), or a reduction in its expression (thalassemia and HbE). Consequently, normal HbA, which is an $\alpha_2\beta_2$ tetramer, is absent and substituted by the mutated hemoglobins, containing an $\alpha_2\beta_2^{Mutated}$ tetramer. Ultimately, the clinical phenotype is caused by the relatively high amounts of the $\alpha_2\beta_2^{sickle}$ tetramer, which allows hemoglobin polymerization and, in turn, leads to vasoocclusion, the hallmark of all sickle cell disorders. In this chapter, we will discuss clinical and laboratorial aspects of the compound sickle cell disorders SC, SD and Sβ thalassemia. In this book, sickle cell anemia is a synonymous for the homozygote state and it is approached elsewhere.

Keywords Genotype • Hemoglobinopathy SC • Hemoglobinopathy SD • Sβ thalassemia

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13.1 Hemoglobinopathy SC

Despite the high prevalence of Hemoglobin SC disease, this hemoglobinopathy is often regarded as being clinically milder than homozygotic sickle cell anemia (HbSS) and very little is known specifically regarding this condition. After Hb SS disease, hemoglobinopathy SC is the most frequent sickle cell disorder worldwide. Hemoglobin C, as HbS, is also African-derived and is caused by a mutation in codon 6 of the Beta-globin chain; however this mutation changes glutamic acid to lysine, instead of to valine, as occurs in HbS. In HbSC disease, red cells contain approximately 50 % HbS and 50 % HbC; however, the presence of HbC is associated with increased K-Cl cotransport activity, which induces loss of K^+ and intracellular water, in turn facilitating the polymerization of HbS (Nagel et al. 2003), Thus, HbSC carriers suffer from acute episodes of vaso-occlusion and a number of complications that are secondary to chronic disease. While hemoglobinopathy SC is generally believed to be a clinically milder disease, compared to sickle cell anemia (homozygotes), it presents a higher frequency of proliferative retinopathy.

13.1.1 Epidemiology

Hemoglobin C is an African-derived mutation and reaches a frequency of 20 % in northern Ghana and Burkina Faso. Recently, genotypic data for sickle cell disease (Saraf et al. 2014) showed that the HbSC genotype ranges from 4 to 12 % of sickle cell diseases in Nigeria and Senegal, but reaches 49.6–92.2 % in Burkina Faso, located in northwestern Africa, bordering Benin. In the Americas and UK, the HbSC genotype ranges from 17.8 to 24.3 % of the total sickle cell disease patient population. Curiously, in Brazil, some regions in the North of the country and in the Northeast of the state of Minas Gerais present an equal incidence of HbSS and HbSC among newborns, reaching 0.1 % of neonates (Fernandes et al. 2010).

13.1.2 Clinical Data

Growth and Development In the Jamaica cohort, the height and weight of HbSC children was found not to differ from normal controls, from birth up to 5 years of age. Anthropometric measurements in 103 HbSC (47 male and 56 female) adult patients from our center (Hematology Center, University of Campinas, Brazil) show a median body mass index (BMI) of 25.9 (min–max; 18.8–46.6), median weight of 68 kg (39–109.2) and median height of 165 cm (143–183), which are similar those of the Brazilian population in general (http://www.ibge.gov.br/home/estatistica/populacao/censo2010/default.shtm). Data are presented in Table 13.1.

Painful Episodes In our cohort, at the time of writing, 8.7 % of HbSC patients had experienced at least two pain episodes per year requiring hospitalization. More than

Table 13.1 Clinical data for double heterozygotes for the sickle cell diseases

Diagnosis	HbSC	HbSβ^0	HbSβ$^+$
Number	103	31	15
Female:Male	56:47	19:12	6:9
Age (y)	38 (13–70)	35 (15:53)	37 (23:56)
Weight (kg)	68 (39.0–109.2)	51 (45:64)	69.4 (50–81)
Stature (m)	1.65 (1.43–1.83)	1.62 (1.57–1.69)	1.63 (1.52–1.82)
Body mass index (BMI)	25.9 (18.80–46.64)	19.7 (17.75–24.42)	25.1 (22.47–28.69)
Retinopathy	39.8 %	10.3 %	26.6 %
ACS	16.5 %	20.6 %	57.1 %
Priapism	2.9 %	10.0 %	0 %
Osteonecrosis	24.2 %	10.7 %	26.6 %
VTE	6.8 %	3.57 %	7.1 %
Stroke	3.9 %	6.9 %	6.6 %
Cholecystopathy	32.3 %	66.6 %	46.6 %
Splenic sequestration	2.9 %	0 %	6.6 %
Leg ulcer	2.9 %	7.1 %	0 %
Osteopenia	27.9 %	46 %	10 %
Osteoporosis	8.8 %	0 %	0 %
RBC transfusion	40.7 %	96.5 %	86.6 %
In hydroxyurea therapy	7.8 %	48 %	27 %

Clinical data were collected from cohorts of patients accompanied at the Hemoglobinopathies Clinic, Hematology Center, University of Campinas, at the time of writing. Percentages refer to the proportion of patients that had experienced the referred manifestation or were undergoing specified therapy

ACS acute chest syndrome, *VTE* venous thromboembolism, *RBC* red blood cell

50 % of patients were asymptomatic and had received an incidental diagnosis after routine blood counts, ophthalmological visits or family investigation of a proband with diagnosis of hemoglobinopathy, based on newborn screening. These data are in accordance with those of Platt et al. (1991) who reported an incidence of 0.4 painful episodes/HbSC patient-year, less than half the rate observed in homozygotes.

Complications Complications occur frequently in this population; in our cohort, 64.08 % of patients had experienced either an acute or a chronic clinical manifestation at least once. As shown in Table 13.1, retinopathy was the most frequent complication in the HbSC cohort with a prevalence of 39 %. Osteonecrosis occurred in 25 patients (24 %), whereas acute chest syndrome (ACS) had been experienced by 17 patients (16 %).

Additionally, cerebrovascular accidents had occurred in four patients (3.89 %); one patient had had a transient ischemic stroke with normal cerebral angiography and three patients had had an ischemic stroke. One patient in follow up and undergoing a transfusion program was uneventful. Other thromboembolic events occurred in 6.8 % of patients, including three patients with pulmonary thromboembolism, two of which occurred during labor or delivery. 32.3 % of patients presented

cholecystopathy and only 2.9 % of patients developed other complications such as leg ulcers or priapism. Auto-splenectomy occurred in 45 % of our patients, corroborating data from Lane et al. (1995). Spleen enlargement had occurred in the remaining patients and, in three of them, at least one episode of splenic sequestration was noted. Osteopenia occurred in 27.9 % and osteoporosis in 8.8 % of the patients, where frequencies were lower than those in homozygotes (57 % with osteopenia and 24.5 % with osteoporosis) (Baldanzi et al. 2011). Our patients underwent at least one echocardiography every 2 years, which showed parameters suggestive of mild or moderate pulmonary hypertension in 5.8 % of the patients and left chamber diastolic dysfunction in 7 %. Hypertension was detected in 22 % of the patients, where two thirds of these individuals were older than 50 years old. In an important review published by Saraf et al. (2014), data regarding pulmonary and cardiac complications in HbSS, HbSC and HbSβ^+ thal patients are discussed (see Table 13.2).

In our HbSC cohort, liver complications were uncommon and appeared only in very severe cases. Two female patients developed chronic hepatopathy, as detected by ultrasound and liver biopsy. Both had a very severe phenotype, with basal hemoglobin levels of above 12 g/dL and were submitted to frequent phlebotomy due to pain. One of these patients (currently 58 years old) developed a cerebrovascular accident (CVA) and is on a red blood cell (RBC) transfusion program. We presume that the sickling process was the primary cause of liver disease in this patient, as we could not find any signs of hepatitis virus, cholelithiasis, clinical hemosiderosis, alcoholism or diabetes. The other patient had hepatitis C and died at 47 years due to G-bacteria infection. With regard to liver enzymes, only two female patients presented a mild increase in alanine aminotransferase (ALT; 69 U/L and 81 U/L respectively, normal < 33). Gamma-glutamyl transpeptidase (GGT) was higher than normal (normal female < 40 U/L and male < 60 U/L) in 25 % of the patients. Conjugated bilirubin was abnormal (above 0.4 mg/dL) in 54 % of the patients, but never reached values above 1.4 mg/dL. One patient had cirrhosis, as detected by ultrasound, and liver steatosis occurred in less than 30 % of the patients. A summary of the laboratorial data is presented in Table 13.3.

Kidney complications also seem to be uncommon in HbSC. In our cohort, the glomerular filtration rate (GFR), measured by clearance of ^{51}Cr-EDTA (Barros et al. 2006) and by serum creatinine values (Cockcroft and Gault 1976), was normal in most patients. Half of our patients older than 50 years old ($n = 9$) showed a mild reduction in GFR (^{51}Cr-EDTA varying from 50 to 77 mL/min/1.73 m^2 and serum creatinine 0.94–1.47 mg/dL). Persistent microalbuminuria (20–200 μg/min) occurred in 15 % of the patients and two patients presented albuminuria (a 37 year old woman with albuminuria of 319 μg/min, serum creatinine of 0.61 mg/dL and GFR of 120 ml/min/1.73 m^2; and a 58 year old woman with albuminuria of 780 μg/min, serum creatinine of 0.94 mg/dL and GFR of 77 mL/min/1.73 m^2).

Pregnancy We evaluated the impact of prophylactic transfusion support in pregnant women diagnosed with HbSC disease. The patients were divided into two groups, according to the type of transfusion support received; 10 women received

Table 13.2 Sickle cell genotype and pulmonary and cardiac complications in the PUSH and Walk-PHaSST cohorts

General clinical manifestations								
	>3 pain episodes PUSH	>3 pain episodes Walk-PHaSST	Leg ulcers PUSH	Leg ulcers Walk-PHaSST	Hb g/dL[a] PUSH	Hb g/dL[a] Walk-PHaSST	LDH U/L[b] PUSH	LDH U/L[a] Walk-PHaSST
Hb SS[c]	16.8 %	31.3 %	1.1 %	22.0 %	8.5 (0.1)	8.6 (0.1)	473 (459–488)	437 (428–446)
Hb SC[d]	10.1 %	27.9 %	0 %	9.0 %	11.5 (0.1)	11.6 (0.2)	279 (260–299)	245 (235–255)
Hb Sβ$^+$-thal[e]	17.6 %	25.9 %	0 %	11.1 %	10.7 (0.3)	11.1 (0.4)	308 (276–344)	245 (213–268)
P	0.3	0.4	0.3	0.002	<0.001	<0.001	<0.001	<0.001

Pulmonary complications								
	Acute chest syndrome PUSH	Acute chest syndrome Walk-PHaSST	O2 sat <95 % PUSH	O2 sat <95 % Walk-PHaSST	TRV ≥2.6 m/s PUSH	TRV ≥3.0 m/s Walk-PHaSST	BNP >160 ng/L PUSH	BNP >160 ng/L Walk-PHaSST
Hb SS[c]	51.3 %	65.3 %	11.1 %	21.6 %	11.7 %	15.0 %	27.7 %	25.8 %
Hb SC[d]	42.7 %	52.7 %	0 %	8.3 %	1.2 %	8.2 %	7.0 %	20.5 %
Hb Sβ$^+$-thal[e]	44.4 %	64.0 %	0 %	0 %	7.1 %	0 %	16.7 %	8.3 %
P	0.17	0.09	<0.001	<0.001	0.013	0.007	<0.001	0.036

Table 13.2 (continued)

Left ventricular size and function								
	LV diastolic dimension Z score PUSH	LV diastolic area (cm^2) Walk-PHaSST	LV mass index (g/m^2) PUSH	LV mass index (g/m^2) Walk-pHaSST	Ejection fraction PUSH	Ejection fraction Walk-PHaSST	Mitral E/E_{tdi} PUSH	LV lateral E/Ea Walk-PHaSST
Hb SS[c]	1.6 (0.6–2.5)	35 (31–40)	94 (78–109)	113 (95–133)	64 (61–67)	61 (58–65)	6.5 (5.7–7.6)	6.4 (5.2–8.1)
Hb SC[d]	1.0 (–0.5–0.7)	29 (26–33)	67 (60–78)	87 (73–108)	64 (61–66)	61 (57–66)	6.1 (5.5–6.9)	6.4 (4.9–7.6)
Hb $S\beta^+$-thal[e]	0.4 (–0.4–1.4)	30 (28–33)	70 (64–90)	84 (69–97)	63 (59–66)	65 (60–68)	5.9 (4.9–6.5)	5.9 (4.8–8.2)
P	<0.001	<0.001	<0.001	<0.001	0.046	0.2	0.004	0.7

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[a]In subjects without recent blood transfusion; adjusted for hydroxyurea

[b]In subjects without recent blood transfusion; adjusted for hydroxyurea, age and study site

[c]PUSH: N = 381 children; Walk-PHaSST: N = 505 predominantly adults

[d]PUSH: N = 90 children; Walk-PHaSST: N = 122 predominantly adults

[e]PUSH: N = 18 children: Walk-PHaSST: N = 27 predominantly adults

Table 13.3 Laboratorial data of double heterozygotes for sickle cell diseases

Parameters	HbSC mean (min–max)	HbSβ^0 mean (min–max)	HbSβ$^+$ mean (min–max)	Reference range
Number	103	31	15	
Hemoglobin, (g/dL)	11.9 (7.27–16.3)	8.7 (5.7–12.2)	9.9 (7.66–13.7)	11.8–16.7
Reticulocytes (×10^9/L)	244 (47.6–485.9)	231.3 (67.5–540)	187.5 (66.78–561.1)	22–139
MVC (fl)	80.8 (60.1–103.3)	77.4 (59.2–101.9)	72.1 (65.9–85.1)	82–98
MCHC (%)	34.3 (30.5–38.4)	33.2 (29.9–35.4)	31.5 (30.5–36.7)	31.6–34.9
Leukocytes (×10^9/L)	8.35 (1.52–16.01)	8.48 (3.99–15.07)	7.4 (3.01–16.01)	3.7–11.1
Neutrophils (×10^9/L)	4.55 (1.47–10.33)	4.0 (2.03–7.96)	3.73 (0.84–6.7)	1.5–7.5
Lymphocytes (×10^9/L)	2.75 (0.9–5.96)	2.86 (0.96–5.97)	2.0 (0.99–5.5)	1.0–3.5
Monocytes (×10^9/L)	0.45 (0.06–1.04)	0.45 (0.1–0.95)	0.32 (0.6–0.84)	0.2–0.92
Eosinophils (×10^9/L)	0.25 (0–0.67)	0.24 (0–1.02)	0.23 (0.01–0.6)	0.02–0.67
Platelets (×10^9/L)	319 (73–644)	418 (98–818)	174 (74–644)	130–400
HbF (%)	1.0 (0.2–5)	7.6 (1.3–24.9)	2.9 (0.3–6.6)	
Ferritin (ng/mL)	209.4 (8.97–1059)	415.7 (64.53–654.8)	316.1 (22.88–1955)	13–400
Serum iron (μg/dL)	81 (26–125)	114.5 (44–243)	70.5 (39.92)	30–160
TIBC (μg/dL)	277.5 (164–358)	230 (172–338)	273 (214–325)	228–428
Transferrin saturation (%)	29.4 (8.42–52.61)	47.52 (16.54–95.34)	27.03 (13.13–42.99)	
Lactate dehydrogenase (U/L)	471 (257–819)	612 (328–932)	469.5 (260–1050)	<480
Serum creatinin (mg/dL)	0.73 (0.39–1.16)	0.57 (0.32–0.96)	0.69 (0.48–1.1)	F < 0.9; M < 1.2
Clearance ^{51}Cr EDTA (ml/min)	98.5 (47–141)	105 (81–151)	106.6 (54.6–157)	<103.4 ± 15
Microalbumin (μg/min)	6.22 (0.78–25.7)	5.85 (1.99–41.8)	5.3 (1.99–9.3)	<30
AST (U/L)	25 (13–48)	33.5 (16–73)	27 (14–61)	<40
ALT (U/L)	19 (8–40)	22 (8–70)	23 (12–54)	<41
GGT (U/L)	27.5 (5–97)	27 (9–69)	42 (19–98)	5–61
Alkaline phosphatase (U/L)	67 (20–150)	83 (41–180)	86 (42–206)	35–129
Conjugated bilirubin (mg/dL)	0.5 (0.2–0.87)	0.6 (0.25–0.9)	0.66 (0.38–0.87)	<0.3
Unconjugated bilirubin (mg/dL)	1.0 (0.3–1.53)	1.2 (0.7–2.4)	0.9 (0.73–5.1)	<0.9

Clinical data were collected, at the time of writing, from cohorts of patients accompanied at the Hemoglobinopathies Clinic, Hematology Center, University of Campinas

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* gamma glutamyl transpeptidase, *TIBC* total iron binding capacity

prophylactic erythrocytapheresis or manual exchange transfusion at 28 weeks of gestation, and 14 received transfusions only on demand, due to acute complications, or no transfusions at all. Our results indicate higher frequencies of SCD related complications in the group of women who had not received prophylactic transfusion support (35.7 % versus 10 % in the erythrocytapheresis group). The complications were also more severe in the latter group, including all cases of acute chest syndrome. Statistical difference was observed concerning gestational age at birth (38.7 weeks in the transfusion group versus 34.4 weeks, $p=0.037$), with a higher frequency of preterm births in the non-transfused group (69.23 % versus 30 % in the transfusion group). Thus, we observed a clear reduction in unfavorable outcomes in patients receiving prophylactic transfusions, probably reflecting better maternal and fetal conditions.

Survival Median survival of HbSC carriers is higher than that of HbSS individuals. Recently, Elmariah et al. (2014) reported that the median survival for HbSC is 66 years and 58 years for HbSS. Elevated white blood counts, lower estimated glomerular filtration rates, proteinuria, higher frequency of pain crises, pulmonary hypertension, cerebrovascular events, seizures, stroke, sVCAM-1, and short-acting narcotics use were significantly associated with decreased survival.

13.1.3 Laboratorial Data

Red Blood Cells HbSC blood smears show very few sickle cells, however many target cells are easily identified, as well as dense and microcytic cells. The low solubility of HbC induces intraerythrocytic crystal formation that may also be identified in the blood smear. Deformability of HbSC cells is lower than normal (Serjeant and Serjeant 2011a; Nagel and Steinberg 2009) and measurements by optical tweezer (Fontes et al. 2011; Brandão et al. 2003) demonstrate a huge heterogeneity; however, many cells show a very low elasticity, with elasticity being even lower than that of RBC of HbS homozygotes or patients with hereditary spherocytosis (Fig. 13.1). Recently, Mozar et al. (2015) reported increased activation of nitric oxide synthase in the RBC of HbSC patients, associated with increased RBC nitrite concentration, reflecting RBC-NOS dependent NO production.

Hematology Data Reticulocytes are usually mildly increased and leukocytes and platelets show normal values and distribution, except in patients with splenomegaly, who may have reduced neutrophil and platelet numbers due to sequestration. Hematological data are presented in Table 13.1.

Alpha thalassemia is common in African-derived populations, especially in populations from West African; therefore HbSC coexistence is expected. In our cohort, heterozygous alpha thalassemia occurred in 15.5 % of HbSC patients and three patients (2.91 %) were homozygotes. The effect of alpha thalassemia on clinical severity of HbSC is unknown; however, a mild clinical course in a 86 year old patient carrying both hemoglobinopathies has been reported (Rodgers et al. 1986).

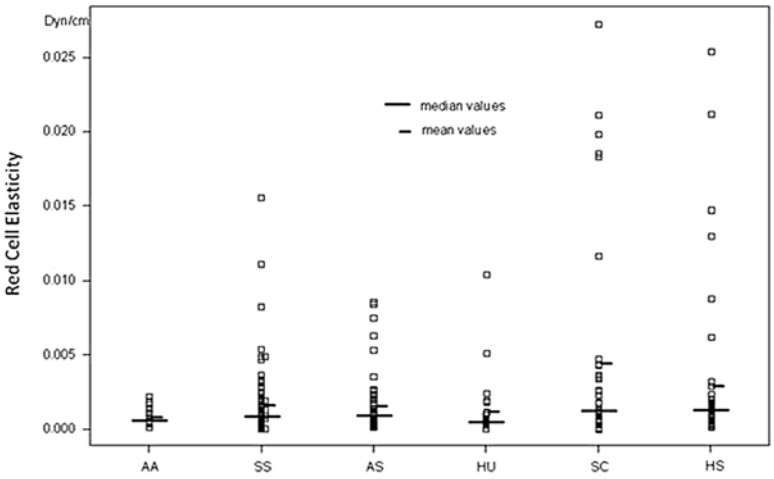

Fig. 13.1 Red cell elasticity (Dyn/cm), as measured by optical tweezers, in healthy controls (AA), and individuals with sickle cell anemia (SS), sickle cell trait (AS), sickle cell anemia on hydroxyurea therapy (HU), HbSC disease and hereditary spherocytosis (HS). Adapted from Brandão (2005)

In our cohort, disease was very severe in two of the alpha-thalassemia homozygotes, who presented CVA, pulmonary thromboembolism (PTE), ACS, pulmonary hypertension, osteonecrosis and retinopathy.

Haplotype Most HbC carriers present haplotype CI, nevertheless CII and CIII as well as atypical haplotypes can be found in a minority of patients. The association of these haplotypes with HbS haplotypes does not modify the hematological characteristics of the patients (Nagel and Steinberg 2009). In our cohort, Haplotypes CII, CIII and atypical, together, were found in less than 10 % of the HbSC patients.

Hemoglobin Distribution As mentioned above, red cells contain equal amounts of HbS and HbC, probably as a result of both abnormal chains competing similarly for alpha-globin chains. HbA2 and HbF are produced in normal amounts; however some patients exhibit a mild increase in HbF, probably related to the genetic background of HbS. In our cohort, HbF values were mostly normal and the median values in our sample were 1 %.

Hemolysis Hemolysis is known to be lower in HbSC, compared to HbSS homozygotes, and the severity of the disease is mainly attributed to high blood viscosity due to high hematocrit values. LDH has long been considered a clinical marker of intravascular hemolysis, which could contribute to complications associated with sickle cell disease (Kato et al. 2006). As such, we investigated associations between LDH and markers of hemolysis and organ dysfunction in our population of 103 patients with HbSC disease. LDH was positively correlated with markers of hemolysis and correlated significantly with reticulocyte counts, but was inversely correlated with haptoglobin levels (Table 13.4). Among patients who did not have complications,

Table 13.4 Correlations between LDH levels and hemolytic parameters in HbSC disease

	Number of patients	rho	*p*
Hemoglobin	85	–0.014	0.901
Reticulocyte count	80	0.471	<0.001
Haptoglobin	69	–0.323	0.007
Indirect bilirubin	57	0.059	0.661

Data collected, at the time of writing, from HbSC patients accompanied at the Hemoglobinopathies Clinic, Hematology Center, University of Campinas. Spearman's rank test

the median LDH concentration was 449 UI/L [257–603 UI/L]. This differed significantly from the median LDH concentration in patients with complications; 479 UI/L [322–2283 UI/L] ($p = 0.012$). Interestingly, an association was observed between LDH and platelet counts (*rho* 0.304, $p = 0.005$). These findings could be related to disease severity, as inflammation may induce thrombocytosis (Griesshammer et al. 1999); however, the high platelet count in our cohort was correlated with auto-splenectomy.

13.1.4 Association between LDH and Clinical Manifestations in HbSC

Although the intensity of hemolysis, as assessed by plasma LDH levels, is expected to be lower in HbSC than in HbSS patients, we investigated this specific parameter and analyzed its correlation with HbSC complications. We found a significant difference between median LDH levels in the subgroups with or without retinopathy and venous thromboembolism (VTE) (451.5 IU/L [257–1816] versus 537 IU/L [354–2283], $p = 0.03$ and 461 IU/L [257–1816] versus 664 IU/L [389–2283] $p = 0.018$, respectively). To further investigate the application of this marker, we determined the associations between LDH levels and complications. ROC curve analysis showed that for retinopathy, LDH > 535 UI/L had a sensitivity of 51.3 % and a specificity of 76 % [95 % confidence interval: 51.8–75.5 %], $p = 0.013$ and OR: 3.3. For VTE, LDH > 606.5 UI/L had a sensitivity of 71.4 % and a specificity of 84.6 % [95 % confidence interval: 55.3–99.2 %], $p = 0.003$ and OR = 13.1. For stroke, LDH > 614.5 UI/L had a sensitivity of 75 % and a specificity of 85.2 % [95 % confidence interval: 38.7–100 %], $p = 0.016$ and OR = 16.3. Finally, for leg ulcer, LDH > 668.5 UI/L had a sensitivity of 100 % and a specificity of 89 % [95 % confidence interval: 83.64–96.85 %], $p = 0.002$ and OR = 2.8 (Fig. 13.2). When these four complications were grouped together, LDH > 535.5 UI/L had a sensitivity of 51.2 % and specificity of 78.6 % [95 % confidence interval: 52.09–75.96 %], $p = 0.007$ and OR = 3.78 (Fig. 13.3).

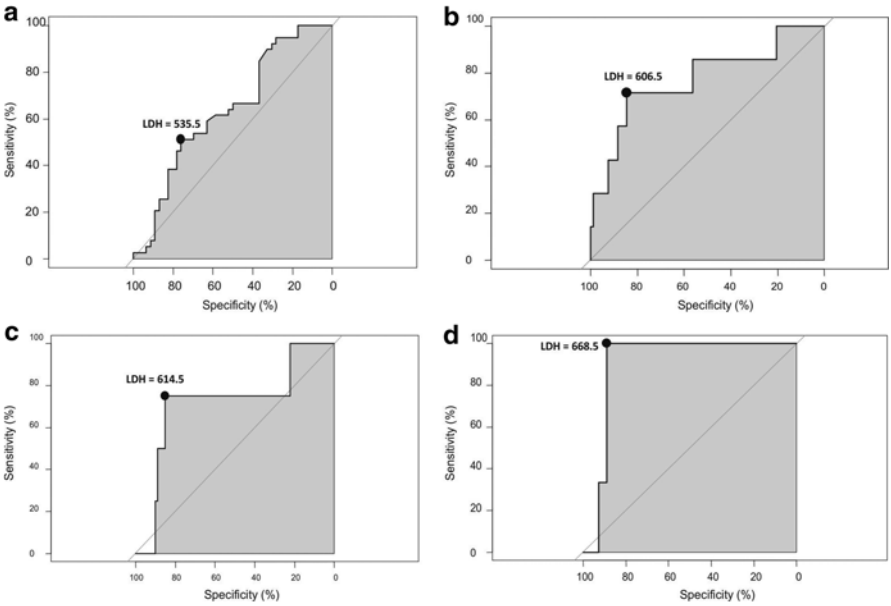

Fig. 13.2 Receiver operating characteristic (ROC) analysis using plasma lactate dehydrogenase levels as a parameter for the prediction of (**a**) stroke, (**b**) venous thromboembolism, (**c**) leg ulcer and (**d**) retinopathy in HbSC patients accompanied at the Hemoglobinopathies Clinic, Hematology Center, University of Campinas

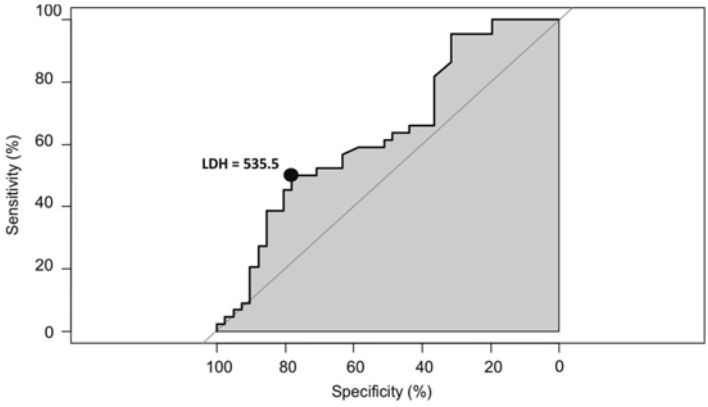

Fig. 13.3 Receiver operating characteristic (ROC) analysis using plasma lactate dehydrogenase levels as a parameter for the prediction of grouped complications (stroke, venous thromboembolism, leg ulcer and retinopathy) in HbSC patients accompanied at the Hemoglobinopathies Clinic, Hematology Center, University of Campinas

13.1.5 Elevated Hypercoagulability Markers in Hemoglobin SC Disease

While an increased risk for thromboembolic events in SC disease has been related (Stein et al. 2006; Novelli et al. 2012), there is a lack of studies evaluating hemostatic alterations in this population. We described a cross-sectional observational study to evaluate coagulation activation markers in adult SC patients, in comparison with SS patients and healthy controls. A total of 56 SC and 39 SS patients were included in the study, all in steady state, and 27 healthy controls. None of the patients were in use of hydroxyurea. HbSC patients presented a significantly upregulated relative expression of *tissue factor*, as well as elevations in thrombin-antithrombin complex and D-dimer, in comparison to controls ($p < 0.01$). Furthermore, HbSC patients presented lower *tissue factor* expression, and thrombin-antithrombin complex and D-dimer levels, when compared to SS patients ($p < 0.05$). Endothelial activation (soluble thrombomodulin and soluble vascular cell adhesion molecule-1), and inflammation (tumor necrosis factor-alpha) markers were both significantly elevated in HbSC patients when compared to controls, being as high as the levels seen in HbSS. Overall, in HbSC patients, higher hemolytic activity and inflammation were associated with a more intense activation of coagulation, and hemostatic activation was associated with two very prevalent chronic complications seen in HbSC disease; retinopathy and osteonecrosis. In summary, our results demonstrate that HbSC patients present a hypercoagulable state, although this manifestation was not as intense as that seen in sickle cell anemia (Colella et al. 2015).

13.1.6 Blood Cell Transfusion and Alloimmunization in HbSC Disease

Overall, patients with HbSC disease have a milder clinical course with a later onset of symptoms. Despite fewer episodes of acute chest syndrome (ACS) and vaso-occlusive crisis (VOC), the incidence of avascular necrosis, retinopathy, and pregnancy-related complications may be high in patients with HbSC. Furthermore, disease severity presents marked variability and some patients with HbSC disease have the same amount of complications as those patients with HbSS disease.

RBC transfusion remains an essential part of the management of patients with SC hemoglobinopathy. Despite the benefits, this procedure increases the risk of serious hazards related to transfusions such as delayed hemolytic transfusion reactions and alloimmunization. Alloantibody formation against RBC antigens is a major complication associated with RBC transfusions in patients with sickle cell disease (which comprises HbSC disease). The alloimmunization rate in this population ranges considerably; dependent primarily on the extent of minor RBC antigen matching and exposure frequency, and the development of multiple alloantibodies is not uncommon, often delaying the location of compatible RBCs.

There are few data in the literature reporting on alloimmunization rates in HbSC disease, specifically. Rosse et al. (1990) found no significant difference between the proportion of alloimmunized HbSS patients (13.1 %) and the proportion of alloimmunized non-HbSS patients (9.1 %) ($p=0.07$). Studies have reported that in the United Kingdom (UK), United States (US) and Kuwait, rates for alloimmunization in SCD patients are 18–76 % with ABO and D matching alone (Davies et al. 1986; Ambruso et al. 1987; Vichinsky et al. 1990; Olujohungbe et al. 2001; Aygun et al. 2002; Castro et al. 2002; Sakhalkar et al. 2005; Ameen et al. 2009), 5–11 % with additional limited phenotype matching for C, E, and K antigens (Sakhalkar et al. 2005; Vichinsky 2001), and 0–7 % for extended minor RBC antigen matching beyond C, E, and K (Tahhan et al. 1994; Lasalle-Williams et al. 2011). In Jamaica and Uganda, alloimmunization rates in SCD patients are even lower: 2.6–6.1 % with ABO and D matching alone (Olujohungbe et al. 2001; Natukunda et al. 2010). The lower incidence of RBC sensitization in these reports is probably influenced by low transfusion burdens as well as homogeneity between recipients and donors of African origin, compared to the UK and US, where donors of African descent represent a minority (Osby and Shulman 2005).

The rate of alloimmunization in our HbSC patients is 21.3 % which is apparently lower than the rate in HbSS patients (32.7 %); however with no statistical difference ($p=0.13$). Despite the current employment of extended phenotyping, we believe that several factors contribute to this result, including transfusions in other services that do not employ the use of the extended phenotype and the age of the patients who were alloimmunized during childhood when only ABO and RH typing were used. Finally, the high rates of Rh gene variants, which are not identified in conventional serological tests, may also have contributed to this index.

13.1.7 Diagnosis

The diagnosis of HbSC is based on the identification of hemoglobin S and hemoglobin C, in equal amounts, in the red cells. They are both easily identified by electrophoresis, isoeletric focusing or HPLC. However, since other hemoglobins may run in the position of HbC, it is important to differentiate them by acid citrate agar. Moreover, in alkaline electrophoresis, HbA2 and HbC migrate in the same region however, the amount of HbA2 is usually below 5 %, thus, when more than 40 % is observed in the HbA2 position, we presume that it is HbC.

13.2 Sβ Thalassemia

The occurrence of Sβ thalassemia is dependent on the distribution and prevalence of both alleles in a given region. Beta-thalassemia is very prevalent in individuals of Italian, Greek and Mediterranean region descent. As such, Sβ thalassemia is more frequent in areas where miscegenation of African descendants and descendants from

these populations occur and can be more prevalent than HbSS in homozygosis in some parts of Greece; however in the Americas, beta thalassemia gene frequency is lower than 0.005 (Saraf et al. 2014; Christakis et al. 1990; Serjeant and Serjeant 2011b).

In patients with $S\beta^0$ thalassemia, the relative amount of HbS inside the RBCs is comparable to that observed in homozygotes and, as such, the phenotype of the disease is similar to that of HbSS. Conversely, the $S\beta^+$ phenotype may vary depending on the expression of HbA. Thus, the amount of HbS in RBCs may be similar to that observed in HbSC, with Hb levels of higher than 9 g/dL, splenomegaly and mild hemolysis. Additionally, the $S\beta^+$ phenotype may be even better if more than 20 % HbA is synthesized. This variation in hemoglobin synthesis is related to the mutational profile of the beta-thalassemic allele. Data from $S\beta^0$ and $S\beta^+$ patients seen in our clinic are shown in Tables 13.1 and 13.3. A comparison of hematological data for Sβ thalassemia individuals from the northeastern region of Brazil is shown in Table 13.5. In our cohort of S beta thalassemia patients (Table 13.1), $S\beta^0$ patients (compared to $S\beta^+$ patients) presented a significantly lower weight (median, 51 kg vs 69.4 kg; $p=0.002$), BMI (median 19.7 vs 25.1; $p<0.001$), lower densitometry values for lumbar spine (median −0.8 vs 0.95; $p=0.01$) and femoral neck (median −0.2 vs 1.2; $p=0.015$), hemoglobin levels (8.7 vs 9.9 g/dL, $p=0.005$), higher platelet number (418 vs 174×10^9/L; $p=0.021$), HbF (7.6 vs 2.9 %, $p=0.001$), serum iron (114 vs 70 μg/dL; $p=0.014$) and transferrin saturation (47.5 vs 27 %; $p=0.004$). These differences are easily understood, since the absence of HbA in the RBC may lead to greater anemia, lower growth, more hemolysis and consequently more osteoporosis/osteopenia and more iron absorption, which would increase transferrin saturation. Moreover, severity of sickle cell disorder is also related to early auto-splenectomy, which causes increased platelet number.

13.2.1 Diagnosis

The diagnosis of Sβ thalassemia is based on the presence of HbS and increased Hb A2 in a patient with low MCV and MCH. The amount of HbA varies according to the molecular defect of the βthal allele.

13.3 Hemoglobinopathy SD

SD disease is a rare sickle cell syndrome, characterized by compound heterozygosity for HbS and HbD. HbD Punjab or Los Angeles is the result of a mutation in codon 121 of the beta-globin chain, which substitutes glutamic acid for glutamine. The glutamine residue facilitates HbS polymerization and patients with both alleles exhibit vaso-occlusion and hemolytic anemia. Both hemoglobins have the same electrophoretic behavior at alkaline pH; however the solubility test, acid pH electrophoresis, HPLC and isoelectric focusing distinguish SD from homozygotes. Data from two patients with HbSD are shown in Table 13.6.

Table 13.5 Hematological data of patients with sickle cell disease

	SS n=20	SC n=20	S/β-IVS-I-6 n=18	S/β-IVS-I-5 n=16	S/β-Cd39 N=12	Reference range
Male:Female	10:10	8:12	9:9	7:9	6:6	
Age (y)	30 (23–39)	28 (18–55)	28 (20–28)	24 (18:35)	34.5 (24–59)	
RBC (10^6/mm^3)	2.46 (1.94–3.41)	4.05 (3.29–5.58)	5.10 (4.64–5.61)	3.64 (2.72–4.56)	3.55 (2.74–4.11)	3.9–6.0
Hb (g/dL)	7.4 (6.3–8.8)	10.9 (9.4–13.2)	11.6 (10.1–12.8)	7.4 (6.3–9.2)	7.8 (6.0–9.6)	11.8–16.7
VCM (fL)	94.3 (84.5–107.7)	85.4 (77.7–92.4)	73.3 (67.2–77.2)	68.7 (61.1–76.8)	73.0 (66.2–76.3)	82–98
Ret (%)	8.6 (4.3–15.6)	3.8 (1.5–7.0)	2.7 (1.0–4.4)	8.8 (4.1–20.0)	8.7 (2.6–13)	0.5–2.5
HbS (%)	89.9 (85.7–92.5)	49.5 (48.1–53.1)	67.4 (64.6–70.1)	72.9 (64.0–85.9)	84.5 (72.5–88.7)	
HbF (%)	6.9 (3.4–11)	1.3 (0.3–3.6)	1.8 (0.3–3.9)	17.6 (8.1–24.8)	11.0 (6.8–23.8)	
HbA (%)	–	–	26.3 (23.3–28.3)	5.2 (3.5–8.7)	–	

Data collected from cohorts of patients accompanied at the Hemoglobinopathies Clinic, Hematology Center of Pernambuco, Brazil (Hospital de Hematologia da Fundação do Estado de Pernambuco, HEMOPE). Values are expressed as means (minimum–maximum). Definitions of β thalassemia-causing mutations: IVS-I-6, single mutation in the sixth nucleotide of intron I (IVS-1); IVS-I-5, single mutation in the fifth nucleotide of intron I (IVS-1); Cd39, mutation in codon 39 of the β-globin gene. Adapted from Bezerra (2009)

Table 13.6 Clinical and laboratorial data for patients with double heterozygosity for HbS and HbD

Parameter	CASE 1	CASE 2	Reference range
Gender	M	F	
Age	39	38	
Hb (g/dL)	9.5	8.5	11.8–16.7
Reticulocytes ($\times 10^9$/L)	251.7	241.3	22–139
MCV (fL)	104.1	99.3	82–98
MCHC (%)	32.9	33.6	31.6–34.9
Leukocytes ($\times 10^9$/L)	7.5	6.86	3.7–11.1
Neutrophils ($\times 10^9$/L)	2.88	3.23	1.5–7.5
Lymphocytes ($\times 10^9$/L)	3.24	2.76	1.0–3.5
Monocytes ($\times 10^9$/L)	0.76	0.5	0.2–0.92
Eosinophils ($\times 10^9$/L)	0.26	0.24	0.02–0.67
Platelets ($\times 10^9$/L)	346	376	130–400
HbF %	7.5	8.5	
Microalbuminuria (μg/min)	15.4	3.09	<30
Serum creatinin (mg/dL)	0.8	0.52	F < 0.9; M < 1.2
Clearance ^{51}Cr EDTA (ml/min)	94	129	<103.4 ± 15
Lactate dehydrogenase (U/L)	2038	1650	<480
AST (U/L)	57	45	<40
ALT (U/L)	19	17	<41
GGT (U/L)	61	12	5–61
Alkaline phosphatase	62	199	35–129
Conjugated bilirubin (mg/dL)	0.7	0.6	<0.3
Unconjugated bilirubin (mg/dL)	5.1	2.04	<0.9
Osteonecrosis	Yes	Yes	
Retinopathy	Yes	No	
ACS	No	No	
stroke	No	No	
VTE	No	No	
Priapism	No	No	
Leg ulcer	No	No	
Transfusion	Yes	Yes	
Alloimmunization	No	No	
Cholecystopathy	Yes	Yes	
Alpha thalassemia	No	No	
Hydroxyurea	No	No	

Data are from patients accompanied at the Hemoglobinopathies Clinic, Hematology Center, University of Campinas

AST aspartate amino transferase, *ALT* alanine amino transferase, *GGT* gamma glutamyl transpeptidase, *HbF* fetal hemoglobin, *VTE* venous thromboembolism

An epidemiological study carried out at the Federal University of Minas Gerais in Brazil (Orsini et al. 2014) showed an incidence of SD carriers of approximately 0.7 % of all sickle cell disease patients, comprising equally SD-Punjab and SD-Korle Bu. SD-Punjab patients have the same clinical phenotype as that of HbS homozygotes and SD-Korle Bu behaves as a sickle cell trait. The β73 residue mutated in HbD Korle Bu does not reduce HbS polymerization.

13.4 Conclusion

Sickle cell disorders are heterogeneous with regard to clinical and laboratorial data, depending mostly on the genotype. $S\beta^0$ thalassemia patients present similar parameters to those of HbSS homozygotes; while $S\beta^+$ thalassemia, hemoglobinopathy SC and hemoglobinopathy SD have similar phenotypes. However, adults from any of these groups can demonstrate severe complications and early death.

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