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Characterization of a cohort of Angolan children with sickle cell anemia treated with hydroxyurea

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ABSTRACT

Background: Sickle Cell Anemia (SCA) is a monogenic disease, although its severity and response to treatment are very heterogeneous.
Objectives: This study aims to characterize a cohort of Angolan children with SCA and evaluate their response to hydroxyurea (HU) treatment and the potential side effects and toxicity.
Methods: The study enrolled 215 patients between 3 and 12 years old before and after the administration of HU, at a fix dose of 20 mg/kg/day for 12 months.
Results: A total of 157 patients started HU medication and 141 of them completed the 12-month treatment. After initiating HU treatment, the frequency of clinical events decreased (transfusions 53.4 %, hospitalizations 47.1 %). The response to HU medication varied among patients, with some experiencing an increase in fetal hemoglobin (HbF) of <5 %. The mean increase in HbF was 11.9 %, ranging from 1.8 % to 31 %. Responders to HU treatment were 57 %, inadequate responders 38.7 % and non-adherent 4.2 %. No clinical side effects related to HU were reported. Hematological toxicities were transient and reversible. Children naïve to HU and with lower HbF reported higher number of hospitalizations caused by malaria infection. During HU treatment, the frequency of malaria episodes did not appear to be affected by HbF levels.
Conclusions: the present study provided a valuable contribution to the understanding of the clinical and laboratory profiles of Angolan children with SCA. These findings support the evidence that the implementation of prophylactic measures and treatment with HU is associated with increased survival in children with SCA.

1. Introduction

Sickle cell anemia (SCA) is a genetic disease transmitted through autosomal recessive inheritance. It results from a mutation in position 6 of the β-globin chain, which causes a substitution of glutamic acid for valine (β6Glu → Val) and produces the S-type hemoglobin (HbS). SCA is one of the most common hereditary diseases in the world, with approximately 300,000 births per year. It is estimated that 75 % of these births occur in Sub-Saharan Africa [1].

Although SCA is a monogenic disease, it can present multiple phenotypic expressions and levels of severity among patients, and even throughout each patient's life [2–4]. Despite the scarcity of information on true SCA mortality in Africa, some studies have concluded that this largely neglected disease has an extremely high mortality rate of between 50 and 90 % in children under 5 years of age, mainly caused by infectious diseases and severe anemia [5]. Malaria not only contributes to mortality but also worsens anemia and other SCA manifestations [6–8]. Other causes of death include acute chest syndrome, stroke, and multi-organ failure [9].

Fetal hemoglobin (HbF) is an effective inhibitor of HbS

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polymerization [10,11]. The concentration of HbF ranges from 1 % to 25 % among patients with SCA [11,12]. Studies have shown that an increase in HbF is associated with a decrease in the number of painful vaso-occlusive events, as well as the number of transfusions and hospitalizations [13,14].

Hydroxyurea (HU) is an HbF-inducing drug that has been approved by both the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA). The clinical efficacy, tolerance, and safety of HU have been well-documented in adult and pediatric patients with sickle cell disease living in high-income countries in Europe and America [15]. However, in Africa, where the prevalence of SCA is high and coexists with malaria, malnutrition, and deficiencies in health systems, there is little robust experience regarding the treatment with HU. Studies have demonstrated that HU can improve patient survival, reduce the intensity and frequency of pain episodes, decrease emergency room visits, shorten hospitalizations, and decrease the number of required transfusions [16–18]. In a multicentric study conducted in Africa, a reduction in malaria cases was also evident, in addition to the clinical benefits described [19]. HU treatment has also been shown to have benefits on neurological events, as evidenced by the reduction in the velocity of blood flow in the cerebral arteries [18,20–22]. In addition to promoting an increase in HbF, HU is also associated with other laboratory benefits, such as an increase in the concentration of total hemoglobin (Hb), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH), as well as a reduction in the number of neutrophils, platelets, reticulocytes, and other hemolysis parameters [19,23–26].

Mild secondary clinical effects associated with HU have also been reported, including headache, gastrointestinal symptoms (nausea, vomiting and diarrhea), rash, and changes in nail color [27,28]. Laboratory toxicity is mainly hematological with mild to moderate neutropenia and thrombocytopenia, both of which are transient and reversed by discontinuing the medication [27,28]. Biochemical alterations (liver enzymes, urea, and creatinine) are minor, reversible, and reported in insignificant numbers [17,28–31].

Some studies have shown that individual responses to HU treatment are highly variable, with HbF levels ranging from 2 % to >30 %, and approximately 25 % of patients do not respond to HU [17,32–34].

In Angola, 1.5 % of newborns in a total of 36,453 children screened during the neonatal period between 2011 and 2013 were found to have SCA [35]. Unfortunately, HU is not universally available for the treatment of Angolan patients with SCA, even for those with severe clinical courses.

Regarding malaria, Angola is an endemic country. Chemoprophylaxis and other measures to control malaria are not commonly implemented. The use of HU in malaria-endemic areas has raised some concerns as it may increase the expression of intercellular adhesion molecule-1 (ICAM-1) in endothelial cells, which could lead to an increase in the cytoadhesion of erythrocytes parasitized with *Plasmodium falciparum* [33]. However, later studies have shown that HU has a beneficial effect and can decrease the incidence of severe malaria [36].

In this study, our aim was to clinically characterize a cohort of Angolan children with SCA and evaluate their response to treatment with HU. We assessed the clinical and laboratory variations in response to HU treatment, including changes in HbF levels, potential side effects and drug toxicity, and treatment adherence. Moreover, we investigated the occurrence of malarial infections during HU treatment. The objective was to treat SCA patients with HU and evaluate the response to the medication for one year, according to clinical and laboratory variation.

2. Material and methods

2.1. Study population

This study enrolled 215 patients between 3 and 12 years old who had a laboratory diagnosis of SCA (confirmed by molecular biology) and were being followed at the Hospital Pediátrico David Bernardino (HPDB) in Luanda and the Hospital Geral do Bengo (HGB) in Caxito. Exclusion criteria included: transfusions within three months prior to enrollment, prior use of HU medication, neoplastic disease, active tuberculosis, HIV infection, and evidence of medullary dysfunction.

2.2. Ethical aspects

Participation in the study was voluntary, and the children's parents or legal guardians were requested to sign a broad written informed consent. The study protocol was approved by the Ethics Committees of the Ministry of Health of Angola (CE. N° 040/2018), and by the Escola Superior de Tecnologia da Saúde de Lisboa (CE-ESTeSL-N°.43-2018).

2.3. Clinical methodology

A prospective study was conducted between 2019 and 2022, consisting of two phases - before (pre-HU) and after the administration of HU (post-HU). However, due to the COVID-19 pandemic, there was a partial interruption in 2020 for seven months (April–October), during which only patients who had already started HU medication were followed up clinically.

During the pre-HU phase, patient characterization and clinical follow-up were performed every three months for a period of six to nine months to obtain clinical and baseline laboratory data. In the first consultation, an anamnesis was conducted to obtain demographic data, age, details of the first clinical manifestation of the disease, number and causes of hospitalizations and transfusions, complications, and a complete physical examination. In the second phase, patients were prescribed HU at a fix dose of 20 mg/kg/day for 12 months. The weekly dose was calculated to adapt the child's weight to the dosage of available capsules, which were 500 mg. Thus, the number of days of medication was calculated as a function of the number of capsules per week. At each appointment, the medication corresponding to the inter-consultation period was offered. During the first six months of medication, patients were seen monthly, and subsequently, the frequency was reduced to quarterly. In this phase, in addition to the variables described above, possible side effects and toxicity of the medication were evaluated through monthly assessment of hematological parameters and assessment of biochemical parameters every three months.

In all follow-up consultations, both in the pre-HU phase and during the medication period, any incidents were recorded, and a physical examination was conducted. Throughout the study period, prophylactic measures were maintained, including the use of folic acid, phenoxymethylpenicillin for children up to 5 years of age, and dietary recommendations.

2.4. Laboratory evaluation

In the first consultation, 5 mL of whole blood was obtained from each child to perform the following tests: (a) hematological evaluation (complete blood and reticulocyte count) using a XT-2000i automatic analyzer (Sysmex Corporation's, Kobe, Japan); (b) biochemical evaluation (bilirubin, lactic dehydrogenase, transaminases, urea and creatinine) using Cobas C111 (Roche Diagnostics', Basel, Switzerland) and BA-88A (Mindray's, Shenzhen, China) analyzers; (c) quantification of HbF by HPLC (Biorad Variant II Hercules, CA, USA); (d) confirmation of SCA diagnosis by molecular biology; and (e) rapid HIV test.

During the pre-HU phase follow-up consultations, which occurred every three months, a complete blood count, reticulocyte count, and the above-mentioned biochemical tests were performed.

To identify possible HU toxicity during the post-HU phase, blood and reticulocyte count tests were performed monthly in the first six months of medication. Every three months, biochemical tests including urea, creatinine, alanine aminotransferase, and aspartate aminotransferase were also conducted. Bilirubin and lactic dehydrogenase were quantified to assess therapeutic efficacy.

Quantification of HbF was performed before the start of HU and every three months after treatment initiation. The average values per period were calculated, and the highest HbF value reached was reported.

For the evaluation of malarial infection during follow-up consultations, it was proposed to obtain two blood samples from each symptomatic patient (fever, headache, worsening of anemia or jaundice, painful crisis) or asymptomatic individual, coincident with the periods of low and high transmission of malaria. The aim was to identify subpatent infections by *Plasmodium* spp. However, due to technical reasons, it was not always possible to obtain these samples. A Rapid Diagnostic Test (SD Bioline) was performed, and if positive, the Giemsa-stained thick smear was observed by optical microscopy. Blood was also stored on filter paper for molecular diagnosis. Parasite DNA was prepared through a saponin/chelex-100 method [37] and subsequently, the detection and identification of *Plasmodium* species were performed by amplification of the genes that code for the smaller subunit of ribosomal ribonucleic acid (ssrRNA) [38], identification of *Plasmodium ovale* [39] was done by nested-PCR according to Fuehrer et al. [40].

2.5. Evaluation variables and operational definitions

The baseline concentration of HbF (pre-HU) for each patient was classified as either low or high based on the obtained quartil. The cutoff point of 7.6 % was adopted, according to the 3rd quartil HbF value, with HbF $\leq$ 7.6 % considered a low level and HbF $>$ 7.6 % considered a high level. To indirectly assess whether patients complied with HU intake, they were asked to show the capsule blisters at each consultation. Medication adherence was assessed taking into account clinical and laboratory parameters.

To evaluate the clinical response to the medication, parameters were compared between the two phases of the study (pre- and post-HU), including the number of pain episodes, transfusions, and hospitalizations.

The evaluation of changes in laboratory parameters was based on the variation in the level of HbF and others such as an increase in Hb, MCV, and MCH, and a decrease in the count of leukocytes, neutrophils, platelets, reticulocytes, and bilirubin. Thus, patients were classified as responders if the increase in HbF was equal to or >5 % in relation to the baseline value or an absolute value of 20 %. Inadequate responders were those with an increase in HbF <5 % but with improvements in other laboratory parameters. Non-adherent patients were those who showed no alterations in the HbF level or other laboratory parameters [41].

Clinical manifestations presumably associated with taking HU were defined as HU side effects, such as nausea, vomiting, abdominal pain, asthenia, and headache [42]. Toxicity was defined by laboratory parameters as Hb values <4 g/dL, severe neutropenia (absolute neutrophil count <500/μL), mild to moderate neutropenia (absolute neutrophil count 500–1249/μL), thrombocytopenia (platelets <80,000 /μL), and reticulocytes <0.2 %. The values of urea, creatinine, and transaminases were also evaluated, and the toxicity criteria were defined considering the reference values.

2.6. Statistical analysis

The data were analyzed using the SPSS program (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp) and were presented using descriptive statistics. The phenotype and laboratorial characteristics were compared using paired sample *t*-tests and qui-square test (p-value $<$ 0.05).

3. Results

3.1. Study population

The study enrolled a total of 215 children, with 141 followed at the HPDB and 74 at the HGB. The age range of participants was 3 to 12 years old, with a mean age of 6.6 $\pm$ 2.5 years. Of the participants, 115 (53.5 %) were female and 100 (46.5 %) were male (Table 1).

3.1.1. Clinical characterization at study entry

Information regarding personal history was obtained by surveying the patients' parents or caregivers.

The age at which patients experienced their SCA first manifestation ranged from 2 to 96 months. Most patients (46.5 %) had their first manifestation between 6 and 11 months of age. Notably, 34 (15.8 %) had their first manifestation before 6 months, and 15 (6.5 %) had it after 36 months. Dactylitis was the most frequent initial manifestation (Table 1).

In 193 (89.8 %) patients, laboratory diagnosis was performed following clinical suspicion, and in 22 (10.2 %) by neonatal screening. All patients had confirmed homozygous SS genotype.

Twenty-six patients had sequelae of complications from the disease, the most frequent being stroke (5.6 %). A total of 146 (67.9 %) patients had received transfusions, with 121 (56.3 %) having an average frequency of 0.1–0.9 transfusions per year. One hundred and sixty-nine (78.6 %) patients had a history of hospitalization, with severe anemia being the most frequent cause (129, 60 %), followed by painful vaso-occlusive crises (91, 42.3 %) (Table 1). Hepatomegaly was observed in 42 children (19.5 %), and the spleen was palpable in 22 (10.2 %) children, of which 13 were older than five years. It may be due to the normal evolution of the spleen in SCA up to 5 years of age, however the cause of splenomegaly after this age has not been investigated. Three children had undergone splenectomy. Despite the presence of hepatomegaly, liver enzyme values were stable, and one patient with splenomegaly had a hemoglobin level below 5 g/dL and another had a platelet count below 80,000/μL on one occasion.

The mean HbF level was 5.8 %, SD $\pm$ 4.1, with a minimum of 0.5 %, maximum of 23.8 %. Three children aged three, four, and seven years had baseline HbF values of 23.8 %, 23.6 %, and 22.3 %, respectively. There was no statistical significance between HbF level and sex, although males had slightly lower values. A correlation was made between the mean baseline HbF level (above or below 7.6 %) and demographic and clinical variables, with statistical significance ($p < 0.05$) observed between age classes at study entry, age at the first manifestation of the disease, number of transfusions, and classes of anemia severity (Table 1).

The mean baseline total Hb value was 7.3 g/dL, SD $\pm$ 0.9, with a minimum of 4.2 g/dL and maximum of 10 g/dL. There was no difference in Hb value between age groups. Among the patients, 3 (1.3 %) had mild anemia, 130 (60.4 %) had moderate anemia, and 82 (38.3 %) had severe anemia, according to WHO anemia severity classes. The MCV varied between 56.4 fL and 113.7 fL, with a mean of 77.2 fL and SD $\pm$ 8.4. The MCH varied between 18.5 pg and 32.1 pg, with a mean of 25.4 pg and SD $\pm$ 2.9. Overall, 66.9 % of patients had microcytosis, and 53.9 % had hypochromia (Table 1).

3.2. Clinical follow-up

3.2.1. Retention of patients in the study

During the study, a total of 73 patients (33.9 %) were lost to follow-up (Fig. 1). In the pre-HU phase, 58 patients (26.9 %) were lost, with 33 (15.3 %) failing to attend scheduled consultations, 21 (9.7 %) unable to be located to resume consultations after a seven-month interruption of the study due to the COVID-19 pandemic, three deaths (two due to malaria and one due to a road accident), and one child withdrawn due to the father's refusal to continue the study (Fig. 1).

A total of 157 patients started HU medication, with 15 (6.9 %) lost during this phase: 13 withdrew from treatment, and two deaths were recorded (one due to malaria at the fifth month of medication, and the other due to severe anemia in an infectious context at the eighth month of medication). Of the patients on medication, 141 completed the 12-month treatment (Fig. 1). The daily dose ranged from 17.2 to 22.7

Table 1
Demographic and clinical characteristics, correlation with HbF level at study entry.

Variables	Total (N, %)	HbF levels	Cutoff point 7.6 %	p-Value
		Low HbF (n,%)	High HbF (n, %)	
Total (n)	215	151 (73.3)	55 (26.7)	
Age group (years)				
≤5	82 (38.1)	49 (32.5)	31 (56.4)	
6–9	95 (44.2)	76 (50.3)	16 (29.1)	**0.006**
>9	38 (17.7)	26 (17.2)	8 (14.5)	
Gender				
Female	115 (53.5)	77 (50.9)	32 (58.2)	0.361
Male	100 (46.5)	74 (49.1)	23 (41,8)	
Age 1st manifestation (mos)				
<6	34 (15.8)	28(18.5)	7 (12.7)	
6–11	100 (46.5)	78 (51.6)	20 (36.3)	
12–23	32 (14.8)	21 (13.9)	9 (16.3)	**0.030**
24–36	34 (15.8)	18 (11.9)	12 (21.8)	
>36	15 (6.5)	6 (3.9)	7 (12.7)	
1st manifestation				
Dactylitis	149 (69.3)	113(74.8)	33 (60)	0.056
Painful crisis	48 (22.3)	25(16.6)	18 (32.7)	**0.009**
Severe anemia	16 (7.4)	13 (8.6)	2 (3.6)	0.235
Stroke	1 (0.5)	0	1(1.8)	0.094
Complications				
None	189 (87.9)	131 (86.8)	52 (94.5)	0.116
Stroke	12 (5.6)	9 (6)	1 (1.8)	0.221
Osteomyelitis	9 (4.8)	8 (5.3)	1 (1.8)	0.450
Hypersplenism	2 (0.9)	1 (0.6)	0	1.000
Femoral head necrosis	1 (0.5)	1 (0.6)	0	1.000
Heart disease	2 (0.9)	1 (0.6)	1 (1.8)	0.464
N° of hospitalizations/ year				
0	37 (18)	22 (14.6)	15 (27.3)	
0.1–0.9	140 (68)	105 (69.5)	35 (63.6)	0.080
>1	29 (14.1)	24 (15.9)	5 (9.1)	
Hospitalizations causes				
Severe anemia	129 (40.7)	99 (41.2)	26 (40.6)	**0.017**
Painful crisis	91 (28.7)	65 (27)	21 (32.8)	0.531
Non-specific infections	48 (15.1)	36 (15)	11 (17.1)	0.561
Malaria	45 (14.2)	37 (15.4)	6 (9.3)	**0.034**
Stroke	4 (1.3)	3 (1.2)	0	0.566
N° of transfusions per year				
0	60 (29.1)	33 (21.9)	27 (49.1)	
0.1–0.9	121 (58.7)	96 (63.6)	25 (45.5)	**0.001**
>1	25 (12.1)	22 (14.6)	3 (5.5)	
Anemia (WHO criteria)[a]				
Moderate	56 (26)	26 (17.2)	(49)	**<0.001**
Severe	159 (74)	12,5 (82.8)	28(51)	
MCV (min-max), fL				
Normocytosis (80–100)	75 (34.9)	48 (31.8)	23 (41.8)	0.447
Microcytosis (<80)	139 (64.7)	102 (67.5)	32 (58.2)	
Macrocytosis (>100)	1 (0.5)	1 (0.7)	0 (0.0)	
MCH (min-max), pg				
Normochromia (26–31.2)	109 (50.9)	71 (47.3)	33 (60.0)	0.073
Hypochromia (18.4–25.9)	105 (49.1)	79 (52.7)	22 (40.0)	

Comparations performed by chi-square test.
Significant values in bold.
[a] Classification of anemia according to WHO criteria (children 5–11 years of age): mild (Hb 11–11.4 g/dL), moderate (8.0–10.9 g/dL), severe (<8 g/dL).

mg/kg.

3.3. Evaluation of HU medication

3.3.1. Adherence, safety and toxicity

The effectiveness of HU medication was evaluated in 141 patients, with 16 cases experiencing two to four weeks of missed medication due to non-attendance at scheduled consultations. By checking the blisters, no capsules were returned. We assumed that patients complied with the prescribed medication dose.

No clinical side effects related to HU were reported. Hematological toxicity occurred in 34 patients (24.1 %), including severe neutropenia (the absolute neutrophil count ranged between 230/μL and 480/μL in three patients), mild to moderate neutropenia (absolute neutrophil count ranged between 700/μL and 1140/μL) in 23 patients, and thrombocytopenia (platelet count between 33,000/μL and 80,000/μL) in eight patients. Of the patients who presented hematological toxicity, five had splenomegaly, one of which presented recurrent splenic sequestration (severe anemia, neutropenia and thrombocytopenia). It should be noted that in six patients, without splenomegaly, moderate neutropenia occurred on two times during medication (in the first and second months of medication and then in the sixth and in one case in the twelfth month).

No serious bacterial infections or associated hemorrhagic manifestations were recorded. All laboratory toxicities were transient and reversible, and treatment was resumed at the same initial dose following a seven-day interruption of HU. Liver enzymes, urea, and creatinine levels did not show significant variations.

3.4. Clinical events related to HU

Comparatively, there was a decrease in the frequency of clinical events from the pre-HU phase to the post-HU phase. Hospitalizations decreased in 53.4 % and transfusions in 47.1 %.

During the medication period, four children experienced complications, with three of them having strokes (one of which was a recurrence), and one child had aseptic necrosis of the femoral head. In two children, the HbF variation was >5 %, while in the other two children, it was <5 %. One child had no variation in total Hb, but there was an increase in corpuscular constants (Table 2).

Out of the 22 children who had splenomegaly at the beginning of the study, one had a history of recurrent episodes of pain, which subsided after starting HU but continued to experience episodes of splenic sequestration with Hb values <5 g/dL. This patient interrupted HU treatment and underwent splenectomy, subsequently showing clinical and laboratory improvement.

3.5. Effect of HU intake on laboratory parameters

3.5.1. Hematological and biochemical parameters

All patients underwent laboratory parameter evaluation while on HU medication. The medication significantly improved HbF level and hematological parameters. Total Hb values and corpuscular constants increased, while values of leukocytes, neutrophils, and platelets decreased. Parameters associated with hemolysis, such as reticulocytes and total bilirubin, showed a significant reduction. LDH also decreased, but not significantly. Liver enzymes, urea, and creatinine, which were used to monitor HU toxicity, showed no significant variation (Table 3).

3.5.2. Fetal hemoglobin

During the pre-HU phase, the mean HbF level was 5.94 % with a standard deviation of 3.91 %, while in the post-HU phase, it increased significantly to 12.19 ± 6.03 % (with a minimum of 2.10 % and a maximum of 31 %). This increase in HbF was observed in all age groups, with statistical significance ($p < 0.001$) for each group. Specifically, in patients aged ≤5 years, the HbF level increased from 7.2 ± 4.5 % to 13.2

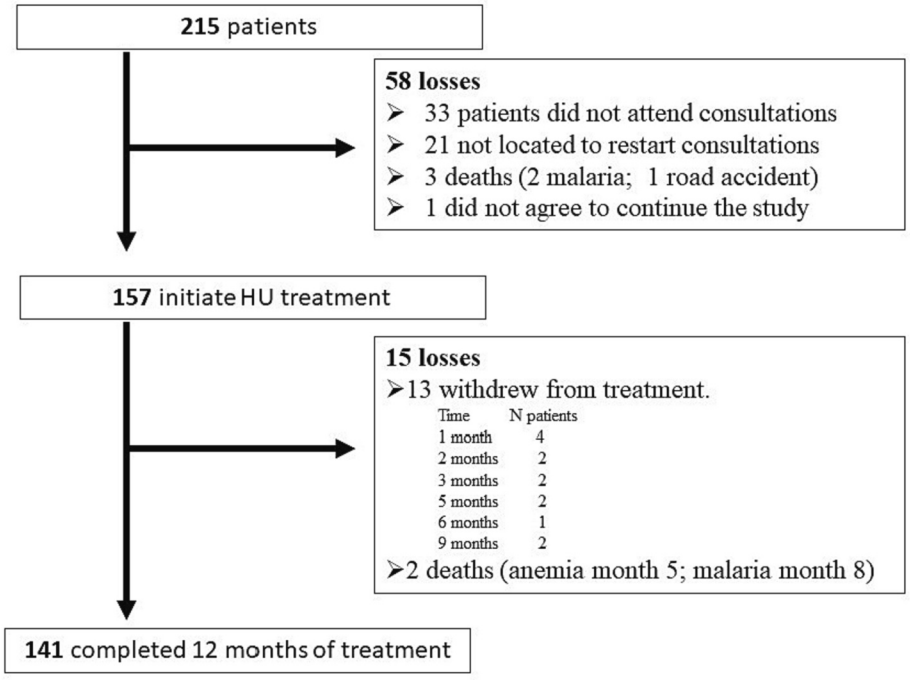

Fig. 1. Patient retention throughout the study.

Table 2
Characterization of children who had complications during the period of medication with HU.

Children	#1	#2	#3	#4
Complications	Avascular necrosis	Stroke	Stroke	Stroke
Age (years)	6	11	9	8
Gender	M	M	F	M
Age 1st manifestation (months)	6	20	6	2
Precedent symptoms	Severe anemia	Osteomyelitis	2 strokes	Severe anemia
Treatment duration (months)	5	2	12	12
Basal HbF (%)	3.4	1.5	5.9	1
Post-HU HbF (%)	10.2	12.8	10.1	1.8
HbF variation (%)	6.7	11.3	4.2	0.8
Hb pre-/post-HU	7.6/8.5	6.4/7.4	6.3/7.5	7.9/7.7
MCV pre-/post-HU	81.4/90.3	82.5/100.5	84.7/93.2	76.7/83.1
MCH pre-/post-HU	26.4/28.8	28.5/34.2	28.7/28.4	26.5/28.0
3.7 kb alpha thalassemia genotype	αα/−α3.7	αα/αα	αα/−α3.7	αα/−α3.7

Table 3
Laboratorial parameters in pre- and post-HU phases.

Laboratory parameters	N	Pre-HU	N	Post-HU	p-Value
		Mean ± SD		Mean ± SD	
HbF	139	5.94 ± 3.91	139	9.9 ± 6.03	<0.001
Hemoglobin (g/dL)	141	7.47 ± 0.89	141	8.00 ± 0.96	<0.001
MCV (fL)	141	76.80 ± 7.69	140	85.02 ± 8.96	<0.001
MCH (pg)	141	25.39 ± 2.91	140	28.88 ± 3.47	<0.001
Reticulocytes (%)	141	9.81 ± 3.74	141	6.80 ± 2.82	<0.001
Leukocytes ($\times 10^3$/μL)	141	13.38 ± 3.82	141	9.91 ± 2.36	<0.001
Neutrophils ($\times 10^3$/μL)	141	5.68 ± 1.89	141	4.21 ± 1.22	<0.001
Platelets ($\times 10^3$/μL)	141	446.90 ± 171.85	141	406.19 ± 122.91	<0.001
LDH (U/L)	141	485.42 ± 184.64	141	476.46 ± 115.88	0.570
Total bilirubin (mg/dL)	140	1.37 ± 1.43	141	1.04 ± 0.50	0.007
Direct bilirubin (mg/dL)	141	0.56 ± 0.91	141	0.56 ± 0.27	0.939
Urea (mg/dL)	134	30.75 ± 18.57	141	33.17 ± 9.49	0.139
Creatinine (mg/dL)	141	0.51 ± 0.60	141	0.43 ± 0.18	0.111
AST (U/L)	141	34.06 ± 16.36	141	35.89 ± 22.76	0.428
ALT (U/L)	141	11.05 ± 9.62	141	17.96 ± 28.33	0.006

Comparisons were made by paired sample *t*-test.

± 6.4 %; in patients aged 6–9 years, it increased from 4.9 ± 3.7 % to 10.8 ± 5.9 %; and in patients aged >9 years, the HbF level ranged from 4.9 ± 3 % to 12 ± 5.1 % after HU treatment. Based on the variation in HbF level, hematological parameters, and bilirubin, patients were classified into three groups: responders (57.4 %, 81 patients), inadequate responders (38.3 %, 54 patients), and non-adherent (4.3 %, 6 patients).

3.6. Malaria infection and HU

A total of 87 cases of malaria were reported during the study, with 40 cases occurring in the pre-HU phase and 47 cases in the post-HU phase. Out of these cases, 16 (18.4 %) required hospitalization, with 10 cases in the pre-HU phase and six in the post-HU phase. However, some patients sought medical attention outside of their scheduled consultations, going to the nearest health unit or hospital emergency departments, making it impossible to collect a sample to confirm the diagnosis or *Plasmodium* species identification.

Molecular diagnosis was performed on a subgroup of 65 children, with 415 dried blood spots analyzed throughout the study period, corresponding to several samples of the same individuals in different stages of the study (54 samples in the pre-HU phase and 361 in the post-HU phase) (Table 4). *Plasmodium* infection was detected in 15 samples from 10 patients in both phases of the study, with two patients testing

Table 4
Malaria Infection as diagnosed by nested PCR and variation of HbF pre- and post-HU.

Patient	Pre-HU	Post-HU/medication time (months)					HbF (%)	
		2	3	4	5	12	Pre-HU	Post-HU
1	*P. falciparum*	Neg	Neg	Neg	Neg	*P. falciparum*	1.1	2.3
2	*P. falciparum*	*P. malariae*	Neg	Neg	*P. malariae*	Neg	5.9	22.3
3	*P. falciparum*	Neg	Neg	Neg	Neg	Neg	10.5	15.5
4	*P. malariae*	Neg	Neg	Neg	Neg	Neg	3.5	8.7
5	Neg	Neg	*P. falciparum*	Neg	Neg	Neg	7.1	6.6
6	*P. falciparum*	Neg	Neg	Neg	*P. falciparum*	Neg	12.4	12.5
7	*P. falciparum*	Neg	Neg	Neg	Neg	Neg	3.9	11
8	*P. falciparum*	Neg	Neg	Neg	Neg	Neg	4.9	8
9	Neg	Neg	Neg	Neg	*P.o. curtisi*	*P.o. curtisi*	6.8	11.8
10	Neg	Neg	Neg	*P. falciparum*	Neg	Neg	1	1.8

positive on two occasions in the post-HU phase. Four patients who had malaria in the pre-HU phase did not experience any new episodes. The frequency of malaria episodes did not appear to change with the increase in HbF, and multiple episodes were observed in cases with a ≥5 % increase in HbF (Table 4). Three *Plasmodium* species were identified: *P. falciparum*, *P. malariae*, and *P. ovale curtisi*, with 11 cases occurring in the high transmission season and four in the low transmission season.

Of the 15 cases with positive *Plasmodium* diagnosis, nine patients had some sort of symptom or alteration. Six patients reported pain, and three patients had a decrease in hemoglobin values, but did not require blood transfusions.

4. Discussion

SCA affects approximately 1.5 % of the population in Angola [35]. Despite HU demonstrated clinical and laboratory benefits worldwide [13,28], particularly in Africa [18], it is still not available as a comprehensive care option for Angolan patients with SCA.

Out of the 215 patients enrolled in this study, 157 (73.02 %) started HU medication, and 141 of them completed the 12-month course. Non-attendance to consultations (25.1 %) resulted in a high number of patients lost, mostly during the pre-HU phase, which may have been influenced by the onset of the COVID-19 pandemic in Angola. The reasons for dropouts were not assessed in this study. In another study, also carried out in an African country, authors identified the frequency and cost of travel as the main reason for loss of follow-up [43].

In our study, we observed that a low baseline HbF level was associated with an earlier age of onset of the disease, a higher number of transfusions, and more severe anemia. Sheehan et al. [25] also reported that a baseline HbF concentration of <10 % was associated with a higher incidence of dactylitis in the first year of life, increased hospitalizations, and transfusions. Additionally, higher levels of HbF (>12 %) were associated with fewer clinical manifestations and a lower risk of chronic organ damage, including stroke, leg ulcers, and priapism [14] In the present study, there was no significant correlation between the baseline HbF concentration and severe complications of the disease. This lack of significant correlation could be attributed to the sample size or other factors that may be involved in the occurrence of major complications, such as, the protective role of alpha thalassemia against stroke, osteomyelitis, spleen, and liver disorders [44,45]; the influence of genetic polymorphisms, in previously identified genes, associated with anemia severity, hemolysis level, hospitalization rate [46] and the incidence of stroke [47].

The medication was well-tolerated, and no clinical side effects presumably associated with HU were reported. The monitoring of medication with laboratory evaluation allowed for the early detection of toxicity markers without clinical implications, which were reversed with a temporary interruption of medication for seven days.

The response to HU medication varied among patients, with some experiencing an increase in HbF of <5 %. The mean of HbF increased to 12.19 %, ranging from 2.10 % to 31.00 %. This variability in HbF response is consistent with previous studies, such as Ware et al. [48], who reported a range of 0.1 % to 26.4 % and a mean of 9.6 %. Polymorphisms in genes that regulate HbF and HU metabolism have been suggested as potential factors contributing to the variation in HbF response to HU treatment [49–51].

From a clinical perspective, there were important improvements during the hydroxyurea treatment phase, namely in the reduction of the number transfusions, number of pain crises reported by the patients and in the number of hospitalizations, proving the beneficial effect of HU treatment. These findings support the evidence that the implementation of prophylactic measures and treatment with HU have been associated with increased survival in children with SCA [28,52].

Four patients experienced serious complications during the medication period, including stroke and necrosis of the femoral head. Of the three children who had a stroke, two occurred after 12 months of medication. Upon evaluating laboratory parameters, we found that the increase in HbF was <5 % and hematological parameters varied. Despite HU medication, classic SCA complications were observed in other studies, such as hepatic vaso-occlusive crisis, avascular necrosis of the femoral head, and recurrent splenic sequestration [31]. Although there is no consensus on this matter, some authors suggest that the duration of medication may correlate with the onset of complications, proposing that longer medication duration may lead to lower frequency of SCA complications [16,31,53].

Regarding malaria, most cases were only reported when patients sought care at other health institutions in urgent situations, making it impossible to obtain a blood sample for timely diagnosis. Of the 15 positive cases confirmed by molecular biology, 11 occurred during the peak transmission season, and of the eight positive cases in the post-HU phase, six were registered before six months of medication. In the study by Olupot-Olupot et al. [8], HU medication was associated with a progressive decrease in the incidence of malaria over the course of medication when the maximum tolerated dose was reached. In cases of malaria, the clinical manifestations associated with the SCA phenotype were episodes of pain and a decrease in Hb levels without the need for blood transfusion [54]. In our study, the highest number of hospitalizations was seen in patients with a low baseline HbF level.

The mortality rate in this study was 2.3 % (n = 5) and was not associated with medication. The deaths occurred in the age group of six to 10 years old and were attributed to malaria (three cases), two of which were in the pre-HU phase. One death was due to anemia in an infectious context in the post-HU phase, and one death was due to an event not related to the disease (a road accident). Patients who died of infectious causes had a low baseline HbF level (HbF ranging from 1.5 % to 5.2 %). It has been previously reported that the level of HbF is inversely proportional to SCA mortality [13]. In Africa the high mortality rate in SCA children is compounded by the prevalence of infectious diseases, especially bacterial and malaria, and this association was also evident in our study. There was a limitation in the study's ability to provide a detailed characterization of clinical episodes due to patients seeking treatment at other hospital units, which made it difficult to

gather precise information about malaria infection and its influence on the SCA phenotype. A more effective approach for future studies would be to collect blood samples on filter paper during acute episodes, even when patients seek treatment at other institutions.

5. Conclusion

The present study provided a valuable contribution to the understanding of the clinical and laboratory profiles of Angolan children with SCA, which can help in the development of guidelines for the use and monitoring of HU in Angola. However, the study also raised important questions about the variability in clinical and laboratory response to HU, as well as the occurrence and recurrence of clinical manifestations in children receiving HU. These questions highlight the need for further studies in Angola to better understand these issues, given the early and severe onset of SCA manifestations in this population. With the anticipated availability of HU in Angola in the short to medium term, it is hoped that more patients will benefit from this medication.

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CRediT authorship contribution statement

Brígida Santos: Writing – original draft, Methodology, Investigation, Formal analysis. **Catarina Ginete:** Methodology, Investigation, Formal analysis, Data curation. **Elisângela Gonçalves:** Writing – review & editing, Methodology, Investigation. **Mariana Delgadinho:** Writing – review & editing, Methodology, Investigation. **Armandina Miranda:** Writing – review & editing, Methodology, Investigation. **Paula Faustino:** Writing – review & editing, Methodology, Investigation. **Ana Paula Arez:** Writing – review & editing, Supervision, Methodology, Investigation. **Miguel Brito:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis.

Declaration of competing interest

There are no interests to declare.

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