Abstract

**Objective:** Evaluating clinical and hematological-clinical parameters of patients with sickle cell anemia (SCA) before and after four years of using hydroxyurea (HU).

**Method:** A retrospective cohort study implementing a quantitative, descriptive and analytical approach developed in two public teaching hospitals located in the Central-West region of Brazil, from November 2010 to October 2011. Data collection was performed through medical records of 32 patients with SCA to assess clinical and hematological parameters before and after HU treatment. The study was approved by the UFMS Ethics Committee under protocol number 1890/2010.

**Results:** All of the 32 patients were homozygous with a mean age in the prescription of hydroxyurea of 19.72±7.58 years, an initial dose of 15.59±4.27 mg/kg/day, and 22.48±5.35 mg/kg/day in the fourth year of treatment. Regarding the use of HU, average values of some hematological parameters presented a significant difference in the fourth year compared to the mean values prior to HU use, such as fetal hemoglobin (14.49±7.52%), red blood cells (2.54±0.38x10¹²/L), hematocrit (25.30±4.03%) and hemoglobin (9.22±3.34g/dL).
Introduction
Sickle-cell anemia (SCA) is a result of an autosomal recessive genetic disorder, with the exchange of two amino acids at position 6 of the β globin chain when glutamic acid (GAG) is exchanged for valine (GTG), resulting in a structural change in the peptide chain [1], characterized by the presence of homozygous hemoglobin S (HbSS) in double heterozygosity (HbAS) with other abnormal hemoglobins (HbSC, HbSD, among others) or in interaction with thalassemias (alpha or beta) [2]. Hemoglobin S (HbS) in deoxygenation suffer physicochemical changes, polymerizing and assuming a sickle shape. Decreased deformability makes blood flow slow, and depending on HbS concentration and deoxygenation, the aggregation of these molecules can lead to vasocclusion. This phenomenon is responsible for acute and chronic manifestations that can affect organs and systems from the first year of being affected and last throughout the life of affected individuals [3].

Episodes resulting from vasocclusion such as painful crises, Acute Thoracic Syndrome (ATS), and stroke/cerebrovascular accident (CVA), among others, cause suffering to the patient and to caregivers/family members [4]. Seeking to mitigate this condition, the therapeutic approach for SCA favors attempts to replace HbS production by fetal hemoglobin (HbF), with the aim to alter spinal proliferation in order to facilitate F cell production kinetics. Fetal hemoglobin (HbF) is predominant in fetal cells and produced from proeritroblasts clones as a result of immature erythocyte precursors, which uniquely activate genes and increase HbF levels [5-7].

The effect of hydroxyurea on the painful episodes of SCA was verified by the Multicenter Study of Hydroxyurea (MSH) in Sickle Cell Anemia, through a randomized double-blinded clinical trial conducted with 299 participants. The MSH related that therapy reduces painful crises and the number of transfusions required [8]. Moreover, hydroxyurea (HU) therapy demonstrated effectiveness in improving hematological parameters in patients [9].

A MSH long-term follow-up study of 17.5 years verified survival of SCA patients treated with HU and found that long-term use of the drug was safe and could reduce mortality [10]. Another retrospective study in adult patients evaluated the effect of HU dosage on the HbF response, organ damage and survival of patients using the drug, and it found that patients should be treated with the maximum tolerated dose before organ damage occurs [11]. Recommendations for health professionals were shown by a panel of experts, including in relation to HU treatment [12].

Considering the published evidence and reports of SCA patients undergoing HU treatment, we realized the need to investigate whether there is a positive relationship between exposure to hydroxyurea and increased fetal hemoglobin, as well as whether there is a positive relationship between the use of hydroxyurea and improvement of hematological parameters. Thus, the following was adopted as the guiding question for this study: What are the effects of immature erythocyte precursors, which uniquely activate genes and increase HbF levels [5-7].

**Conclusion:** Treatment with hydroxyurea showed a significant increase in fetal hemoglobin levels, increased hemoglobin, hematocrit and average corpuscular hemoglobin concentration, with reduced episodes of pain, infection and acute chest syndrome in such a way as to reaffirm its efficiency in treating these patients.

**Keywords**
Hemoglobin; Sickle Cell Anemia; Hydroxyurea.
of using hydroxyurea in relation to the clinical and hematological parameters of patients with sickle cell anemia?

In this perspective, this study aimed to evaluate the clinical and hematological parameters of patients with sickle cell anemia before and after four years of using hydroxyurea.

Method
This is a descriptive and analytical retrospective cohort study with a quantitative approach, developed in two public teaching hospitals located in the center-west region of Brazil from November 2010 to October 2011. Data collection was conducted from the medical records of 32 patients with SCA in order to evaluate the clinical and hematological parameters before and after HU treatment.

A time interval of thirteen years for including participants to be evaluated was established, corresponding to the years between 1998 and 2010. This period was established since it corresponded to the period in which the studied services registered patients treated with HU and provided the necessary information for evaluating the use of this medicine.

Patients with confirmed electrophoretic profile for HbSS with medical indication for HU therapy, a medical diagnosis confirmed by ICD10 D57.0 and D57.1, which correspond to cases of sickle cell anemia with and without crisis, respectively, in addition to undergoing HU treatment for a minimum period of four years were included in the study. Medical records of patients diagnosed with other hemoglobinopathies and patients undergoing HU for a period of less than four years were excluded.

A data collection instrument was developed specifically for systematizing the information extraction necessary for this study. The variables adopted (Table 1) were adapted from the Ministry of Health of Brazil MS/SAS number 55/2010 [13], which establishes the Clinical Protocol and the therapeutic guidelines for sickle cell disease in the country.

In order to evaluate the effect of HU treatment in patients with sickle cell disease, the studied variables were organized into two moments, being before and after therapy. The "before" refers to data recorded in the period one year prior to using the drug, and the "after" corresponds to the fourth year of use. The four-year period was stipulated in this study as a safety margin, considering that the MS/SAS Ordinance number 55/2010 requires a...
minimum interval of two years for evaluating HU treatment [13].

Data were collected in the first semester of 2012. The events were recorded in an Excel® 2010 spreadsheet according to the diagnosis established in the medical records. EpilInfo version 3.4.1 and Bio-Stat 4.0 [14] were used for data analysis, with descriptive analysis and tabular representation of the results.

Student’s t-test for paired samples and Wilcoxon test were used to compare the means of the quantitative variables in the two moments evaluated. Both tests were used after checking the distribution normality by the Kolmogorov-Smirnov Test. A significance level of 5% was adopted.

This study was approved by the Research Ethics Committee of the Federal University of Mato Grosso do Sul under Protocol number 1890/2010, in accordance with the current national legislation for human research.

Results

The mean age of the 32 patients at the start of their HU treatment was 19.72±7.58 years. The mean initial dose of HU was 15.59±4.27 mg/kg/day, and 22.48±5.35 mg/kg/day in the fourth year.

In comparing mean values of hematological parameters before and after HU use, HbF (14.49±7.52%), RBC (2.54±0.38x10¹²/L), Hct (25.30±4.03%) and Hb (9.22±3.34 g/dL) presented significant differences (p<0.001). The mean parameter values in the fourth year for HCM (35.07±4.99pg, p<0.003), PLT (327,029±110,503 μl, p<0.002) and ANC (6,586.53±2,643.90, p<0.03) were also significant. No statistically significant results were found in comparing MCHC and MCV (Table 2).

In comparing the number of episodes resulting from vasoconstriction before and after the use of HU, the following results were observed: Crisis (102 to 72), Infection (48 to 15), Pneumonia (33 to 16) and ATS (Acute Thoracic Syndrome) (12 to 2). The comparison between the number of blood transfusions at both moments presented an occurrence of 10.0±8.0 for 7.0±6.0 procedures (Table 3).

Table 2. Hematologic parameters of patients with sickle cell anemia before and after four years of using hydroxyurea, Campo Grande/MS, Brazil, 2016 (n = 32).

<table>
<thead>
<tr>
<th>Hematologic parameters</th>
<th>Hydroxyureia</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before using</td>
<td>After 4 years of using</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>HbF (%)</td>
<td>7.59</td>
<td>5.22</td>
</tr>
<tr>
<td>He (10¹²/L)</td>
<td>2.26</td>
<td>0.30</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>21.88</td>
<td>3.55</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>8.17</td>
<td>3.15</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>32.88</td>
<td>1.88</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>32.46</td>
<td>3.40</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>101.79</td>
<td>11.96</td>
</tr>
<tr>
<td>Leukocytes WBC (10⁹/L)</td>
<td>12.657</td>
<td>3.986</td>
</tr>
<tr>
<td>PLT (µL)</td>
<td>290.333</td>
<td>106.696</td>
</tr>
</tbody>
</table>

¹: Student’s T-test for paired samples. ²: Wilcoxon Test.

Table 3. Complications due to vasoconstriction in patients with sickle cell anemia before and after four years of using hydroxyurea. Campo Grande/MS, Brazil, 2016 (n = 32).

<table>
<thead>
<tr>
<th>Hematologic parameters</th>
<th>Hydroxyureia</th>
<th>reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before using</td>
<td>After 4 years of using</td>
</tr>
<tr>
<td>CVA</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>ATS</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Infections</td>
<td>48</td>
<td>15</td>
</tr>
<tr>
<td>Cholelithiasis</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>33</td>
<td>16</td>
</tr>
<tr>
<td>Pain crises</td>
<td>102</td>
<td>72</td>
</tr>
<tr>
<td>Priapism</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>N° of transfusions</td>
<td>10</td>
<td>7</td>
</tr>
</tbody>
</table>

*: low frequency. 

This article is available at: www.intarchmed.com and www.medbrary.com
Discussion

The patients’ mean age at the time of HU treatment initiation was 19.72±7.58 years with varying doses, where the mean in the first year was 15.59±4.27 mg/kg/day and 22.48±5.35 mg/kg/day in the fourth year. These data were similar to those found in a prospective cohort study on the effects of HU conducted with SCA patients over 18 years of age administered a mean dose of 21 mg/kg/day, ranging from 10 to 35 mg/kg [15].

The gradual scaling regimen recommended by Ordinance MS/SAS number 55/2010 of the Ministry of Health of Brazil is initially a single dose of 15 mg/kg/day, with a gradual increase not exceeding the maximum tolerated dose of 35 mg/kg/day. The maximum tolerated dose approved by the Food and Drug Administration (FDA) for moderate and severe adult patients is up to 35mg/kg/day [16]. A daily oral dose of 25 to 30 mg/kg was tested and well tolerated by the majority of children aged 5 to 15 years participating in a study conducted in the United States [17].

The presented HbF values were significantly higher in the fourth year of HU treatment (14.49%) compared to the mean of the year before administration of the drug (7.59%). An observational study was conducted investigating 32 patients regarding HU scaling dosage, of which 26 were treated with the maximum tolerated dose (ranging from 10 to 35 mg/kg/day) for two to 38 weeks, and showed an increase in HbF (4.0±2% to 15±6%) among the analyzed laboratory parameters [15].

In response to the drug’s effects, another study revealed that two patients treated with HU had an increase in young fetal cells between 48 and 72 hours, which resulted in a high level of HbF [18]. A multicenter American study evaluated the safety of HU therapy in children aged 5 to 15 years, and as a result 69 children achieved the highest tolerated dose of HU (25.6±6.2 mg/kg/day), resulting in a higher production of HbF (17.8±7.2%) compared to the initial treatment parameter (7.3±4.9%) [17].

In this study, a significant increase in red blood cell count (2.54 10^{12}/L, p<0.001) and hematocrit (25.30%, p<0.001) was found in the fourth year of HU use in comparison to the year before instituting the drug. These results are similar to those described in an English study, which showed that erythrocyte alterations due to the effect of HU cause an elevation of Hb and Hct, in addition to hydrating and improving erythrocyte survival.9 In another study, an increase in MCV and HbF was observed, however with reductions in leukocyte, platelet, reticulocyte, neutrophil and total bilirubin counts [17].

MCV ranged from 32.88g/dL in the year prior to instituting therapy to 33.21g/dL in the fourth year of HU treatment. The same occurred with MCV, which initially presented a result of 99.51fL and 101.79fL in the fourth year; however, none of these had a significant relation when compared to the average of the year prior to instituting the drug, but remained within the reference values of the present study. These data do not corroborate with published evidence, suggesting that MCV is directly related to the increase in HbF [9].

When the MCV and HbF were analyzed together with the HU dosage, the results of this study showed that MCV levels in the fourth year did not have the expected increase. Scientific evidence regarding HU therapy emphasizes that there may be failures during treatment, including the fact of non-monitoring not only regarding medication use, but also regarding requesting of laboratory results in a systematic way, age at the time of indication and/or institution of the drug, ideal dosage, among others [19]. The studies in which the maximum tolerated dose was reached probably registered the best results [9].

In the fourth year, leukocytes were reduced to 11.12×10^9/L (±3.76×10^9/L; p=0.080), but remained within the reference values. In a retrospective study conducted in the US with 383 adult patients with sickle cell anemia between 2001 and 2010, absolute neutrophil count (ANC) was lower in the last consul-
tation for the group receiving HU (4.9%; \(p<0.001\)) [11]. However, it has been concluded that adults should be treated with the maximum tolerated dose of HU, preferably before organ damage.

In the fourth year, neutrophils (7,729.25\%, \(p=0.030\)) and hemoglobin (9.22 g/dL, \(p<0.001\)) values showed a significant relationship when compared to the mean of the year prior to instituting the drug. In an observational study also conducted in the United States, neutropenia (73\%), reticulocytopenia (22\%) and a decrease in Hb concentration (1\%) were detected at the moment of HU dose adjustment [15].

To monitor dose adjustment, neutrophil count, platelet count, reticulocyte count, and hemoglobin level may not be less than 2,000/mm\(^3\), 80,000/mm\(^3\), 80,000/mm\(^3\), and 4.5 g/dL, respectively [5, 13]. Bone marrow suppression may occur, but it is reversible and transient with discontinuation of treatment, and specifically for granulocyte series, it represents a common adverse effect in patients treated with HU. The most frequent adverse events include neutropenia and thrombocytopenia, as well as anemia to a lesser extent [5, 20].

Platelets remained within the reference values when compared to the mean of the year prior to instituting the drug (327,029 to 290,333 μL, \(p=0.002\)). An observational follow-up study originating from MSH evaluated the effects of HU on mortality and morbidity in 233 adults with sickle cell disease between 1996 and 2001. In this study, a subgroup of 63 patients with smaller reticulocyte counts (<250,000/mm\(^3\)) and Hb levels of <9g/dL, also presented platelets within the reference parameter after two years of HU treatment (401,700±146,900 μL) [21].

Pain episodes due to vasoconstriction were reduced after HU treatment (102 to 72). It should be mentioned that the key point of the MSH study was a significant reduction of pain episodes [8]. Improvement in erythrocyte deformity makes erythrocytes more spherical, and morphological changes in conjunction with rheology can represent a potential benefit to patients with sickle cell disease [16]. HU acts from proeritroblast precursors, favoring red cell production with a high level of Hb F [4], and in this way partially inhibits polymerization and avoids falcination in deoxy-HbS conditions with a consequent reduction in painful crises [22].

After four years of HU treatment, lung infections (specifically pneumonia) reduced from 33 to 16 episodes. Splenic function (which is decreased in SCA) is one of the factors that contribute to greater susceptibility to infections. Over time, infections in SCA patients can affect organs from already damaged systems, such as lungs damaged by recurrent pneumonia, kidneys afflicted by urinary infections and bones with osteomyelitis. Bacterial, viral and parasitic infections cause greater morbidity and are difficult to control [3].

ATS and CVA episodes resulting from vasocclusion reduced from 12 to two cases and from four to one, respectively, after HU use. The MSH study demonstrated the efficacy of this drug in reducing pain episodes following UH therapy over placebo [8].

The mean number of blood transfusions in the fourth year of HU use was reduced from 10 to 7 transfusions in comparison to the year before treatment. In the MSH study, 299 severely affected adults with three or more pain episodes/year in the HU group had reduced transfusions (73 to 48, \(p=0.001\)) [8].

Scientific evidence has demonstrated the efficacy of HU in reducing the frequency of painful episodes, improving hematological parameters [13] and toxicity without serious side effects. Therefore, the results of this study have demonstrated that HU therapy remains an option to be offered in order to benefit a larger number of patients with sickle cell disease worldwide.

Reticulocytes were not collected in this study because of the lack of systematic registering in medical records. Thus, hemolysis markers were not analy-
zed, representing a limiting factor of this study, in addition to having data collection from secondary and retrospective sources.

**Conclusion**

The effects of hydroxyurea on hematological parameters demonstrate a significant increase in the level of HbF, improving Hb, RBC, Hct, and MCH concentration and reducing absolute neutrophils. The use of this therapy decreases painful crises, infection (pneumonia) and ATS.

This study considerably contributes to SCA knowledge, as it provides evidence of improved treatment conditions which may lead to effective interventions for patients with sickle cell disease in health services.

It is up to health systems to ensure the use of this drug as a treatment option for patients with sickle cell disease. We suggest future investigations that evaluate the efficacy of HU and the ideal dosage through experimental and epidemiological studies involving large populations.

**References**


International Archives of Medicine is an open access journal publishing articles encompassing all aspects of medical science and clinical practice. IAM is considered a megajournal with independent sections on all areas of medicine. IAM is a really international journal with authors and board members from all around the world. The journal is widely indexed and classified Q2 in category Medicine.