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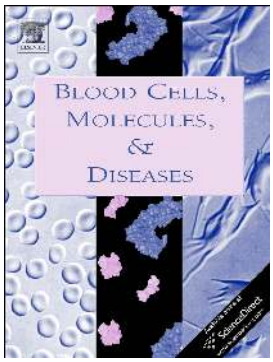

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Real-life experience with hydroxyurea in sickle cell disease: a multicenter study in a cohort of patients with heterogeneous descent

Short title: HU for SCD in a Southern European country

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ABSTRACT

We conducted the first nation-wide cohort study of sickle cell disease (SCD) in Italy, a Southern European country exposed to intense recent flux migration from endemic areas for SCD. We evaluate the impact of hydroxyurea on a total of 652 pediatric and adult patients from 33 Reference Centers for SCD (mean age 24.5 ± 15 years, 51.4% males). Hydroxyurea median treatment duration was 7 years (range: <1 year to 29 years) at a mean therapeutic dose of 18 ± 4.7 mg/kg/day. Hydroxyurea was associated with a significant increase in mean total and fetal hemoglobin and a significant decrease in mean hemoglobin S, white blood and platelet counts, and lactate dehydrogenase levels. Hydroxyurea was associated with a significant reduction in the incidence of acute chest syndrome (-29.3%, p<0.001), vaso-occlusive crisis (-34.1%, p<0.001), hospitalization (-53.2%, p<0.001), and bone necrosis (-6.9%, p<0.001). New silent cerebral infarction (SCI) occurred during treatment (+42.4%, p<0.001) but not stroke (+0.5%, p=0.572). These observations were generally consistent upon stratification for age, descent (Caucasian or African), genotype (βS/βS, βS/β^0 or βS/β^+) and duration of treatment (< or ≥10 years). There were no new safety concerns observed compared to those commonly reported in the literature. Our study, conducted on a large population of patients with different descent and compound state supports the benefits of hydroxyurea therapy as a treatment option. Registered at clinical trials.gov (NCT02709681).

Keywords: hydroxycarbamide, management, real-world, complications.

INTRODUCTION

With the ongoing search for targeted and curative therapeutics for sickle cell disease (SCD) and its manifestations, hydroxyurea remains a cornerstone of conventional management owing to its oral efficacy and low toxicity [1,2]. The disease modifying properties of hydroxyurea were initially attributed to its ability to induce fetal hemoglobin and decrease hemoglobin S polymerization [3,4]. which should theoretically ameliorate downstream pathophysiologic mechanisms, acute and long-term clinical morbidity. Other beneficial effects have subsequently emerged including increasing total hemoglobin levels, decreasing platelet and white blood cell counts, changing expression of adhesion molecules, and nitric oxide generation [1,5,6]. Thirty years of clinical experience through randomized clinical trials and large observational studies established that hydroxyurea is safe and effective in decreasing the frequency of acute complications like painful vaso-occlusive crisis and acute chest syndrome, while also decreasing the need for blood transfusion and hospitalization in SCD adults and children as young 9 months of age [7-13]. Long-term follow-up studies have also established continued benefit as well as reduction in mortality [14-17]. In addition, the initial concerns on HU effects on fertility and carcinogenic potential have not been fully established in patients with SCD and require a long-term follow-up on large cohorts of SCD patients [7-13]. Despite such substantial body of evidence, hydroxyurea is considered an underutilized medication in SCD [1,13,18]. Thus, data from real-life experiences with hydroxyurea remain essential to further illustrate the role of this intervention to practicing clinicians.

Until the last decade, SCD was endemic in Southern Italy (Sicily and Calabria) with limited number of patients spread all over the country due to internal migration. Thus,

data on the epidemiology and clinical profile of SCD in Italy, as Southern European country exposed to intense migration fluxes from areas endemic for SCD, such as the Sub-Saharian countries [19] , have been deeply changed. Such current and future mobility and migration flows, pose considerable new challenges that have to be taken into consideration by member states and EU authorities primarily through collection of data from existing patients.

With this background, the aim of this study was to report the first, real-life experience with the use of hydroxyurea in a large cohort of SCD patients with heterogeneous descent and different compound state.

METHODS

This was a retrospective cohort study of SCD patients attending treatment centers across Italy. All Italian Hematology Centers part of the Italian Society of Thalassemia and Hemoglobinopathies (SITE) and all Pediatric Hematology Oncology Units part of the Italian Association of Pediatric Hematology Oncology (AIEOP) were invited to participate in the study. Invitation was expressed during two meetings of the Working Groups and by a letter. All large Regional Reference Centers (pediatric and adult) for therapy of SCD participate to study. Out of 1638 patients registered at 33 participating centers, 652 (39,8%) patients had received hydroxyurea therapy for some period throughout their disease course and were included in this analysis. The indication for hydroxyurea initiation was 2-3 vaso-occlusive crisis and/or acute chest syndrome in the year prior. Hydroxyurea was initiated at a starting dose of 10 mg/kg/day, and adjusted or escalated according to tolerance. For each patient, retrieved data included demographics (age and gender), origin, genotype, and folic

acid use. The duration of treatment (until discontinuation or death) and average hydroxyurea dose throughout therapy were also recorded. We also retrieved average of all available laboratory values up to three years pre-hydroxyurea and for the period on-hydroxyurea therapy including total hemoglobin level, fetal hemoglobin level, hemoglobin S level, white blood count, platelet count, lactate dehydrogenase level, total and direct bilirubin levels, aspartate and alanine aminotransferase levels, and serum creatinine level. The incidence of new complications pre- and on-hydroxyurea therapy was also retrieved as available from medical records and as defined by internationally recognized criteria [20], including: stroke, silent cerebral infraction, acute chest syndrome, vaso-occlusive crisis, hospitalization, leg ulcers, pulmonary hypertension defined as patients with a tricuspid-valve regurgitant jet velocity ≥3.2 m/s (3.6%) on transthoracic echocardiography further underwent right heart catheterization to confirm the diagnosis of PAH (mean pulmonary arterial pressure ≥25 mm Hg and pulmonary capillary wedge pressure ≤15mm Hg) [21,33,34,35], AVN defined as bone necrosis confirmed by radiographs and in some cases MRI and chronic kidney disease was defined according to the National Kidney Foundation, Kidney Disease Outcomes Quality Initiatives (K/DOQI) guidelines. [22]. For silent cerebral infarction, only patients with magnetic resonance imaging performed within the last ten years were considered to limit the chance of varying imaging methodology. In such patients screening scans were repeated every two years. For acute chest syndrome, vaso-occlusive crisis and hospitalization, aside from incidence the average number of episodes per year (to the nearest integer) were collected up to three years pre-hydroxyurea and for the period on-hydroxyurea. Safety data included adverse events as reported by the treating physician and the

incidence of malignancy or death. Pregnancy incidents and their outcomes were also collected.

The study was approved by the ethical committee of Palermo 2 and the study was registered at clinical trials.gov (NCT02709681).

Statistical analysis

Descriptive data were reported as mean ± standard deviation, median (range), or percentages. Bivariate comparisons of laboratory and clinical data pre- and on-hydroxyurea were done using the paired-samples t-test for means, Wilcoxon matched-pair single-rank test for medians, and McNemar's test for percentages. Spearman's correlation (r) coefficient was used to evaluate correlations between hydroxyurea dose and changes in laboratory parameters. A Kaplan Meier curve was drawn to illustrate survival with and without hydroxyurea therapy. The Cox proportional-hazards regression model was used to estimate the hazard ratio and the 95% confidence interval (CI); the proportional hazards (PH) was checked (test and graphical diagnostics) by means of the scaled *Schoenfeld residuals.* The comparison between HU-treated and non-treated patients was done with the unpaired Wilcoxon test. In the Cox model the group of non-treated patients was compared with a subset of HU-treated patients with the age in the range of 25^{th} and 75^{th} percentile of the age of no treated subjects. . All p-values were two-sided with the level of significance set at <0.05.

RESULTS

Patients' characteristics

A total of 652 SCD patients who had received hyrdroxyurea were included in this analysis. The mean age at the time of hydroxyurea initiation was 24.5 ± 15.0 years (range: 1.0-67.0), with 32.7% of patients being in the pediatric age group (<18 years). There was an equal gender distribution with 51.4% of patients being men. The majority of patients were of Caucasian (64.4%) or African (35.6%) origin; **Supplementary Figure 1** illustrates the origins of patients analyzed in this study. Around half of the patients (46.6%) had a βS/βS genotype while the remaining patients had β^0/βS (28.1%), β^+/βS (22.1%), or other genotypes including βS/βC (3.2%). Patients' characteristics are summarized in **Table 1**.

Hydroxyurea therapy

The median duration of hydroxyurea therapy in the study sample was 7 years (range: <1 year to 29 years). The wide range of treatment duration is related to the retrospective characteristic of this study. In fact, the large part of Caucasian patients started treatment many years ago since 1995. Whereas, pediatric subjects were placed under HU therapy after Baby HUG study [10,13] . The mean therapeutic dose was 18.0 ± 4.7 mg/kg/day (range: 6.0-32.0). The distribution of dose categories was: <10 mg/kg/day (n/N=14/598, 3.2%), 10-20 mg/kg/day (n/N=434/598, 72.6%), and >20 mg/kg/day (n/N=150/598, 25.1%). Folic acid was concomitantly used in 71.3% of patients (n/N=388/448).

Changes in laboratory parameters following hydroxyurea therapy

Changes in laboratory parameters pre- and on-hydroxyurea therapy are summarized in **Table 2**. Hydroxyurea therapy was associated with a significant increase in mean total hemoglobin level (+0.5 g/dL, p<0.001). Hemoglobin increase was ≥1.0 g/dL in 44.5% (n/N=125/490) of patients and was ≥2.0 g/dL in 19.0% (n/N=218/490) of patients. Hydroxyurea therapy was also associated with a significant increase in mean fetal hemoglobin level (+8.0%, p<0.001), while it was also associated with a significant decrease in mean hemoglobin S level (-7.3%, p=0.019). It was associated with a mean increase in mean corpuscular volume (+13.6 fL, p<0.001). Hydroxyurea therapy was also associated with a significant reduction in mean white blood counts (-2.3 $x10^9$/L, p=0.01) and platelets (-44.9 $x10^9$/L, p<0.001). Reticulocyte count also decreased although the change did not reach statistical significance. White blood counts <1.0 x 10^9/L were observed in three patients on hydroxyurea, while platelet counts <150 $x10^9$/L were documented in 77 patients although 19 (24.7%) of them already had counts <150 $x10^9$/L pre-hydroxyurea therapy. Hematological value in according to genotype are summarized in **Table 3**. Mean lactate dehydrogenase levels were significantly decreased on hydroxyurea therapy (-161.3 IU/L, p<0.001), while bilirubin levels remained unchanged. A small but significant reduction was observed in mean aspartate aminotransferase level (-6.9 IU/L, p<0.001), while mean serum creatinine level was comparable pre- and on-hydroxyurea therapy **Table 2**.

Changes in complication rates following hydroxyurea therapy

Hydroxyurea therapy was associated with a significant reduction in the incidence of acute chest syndrome (-29.3%, p<0.001), vaso-occlusive crisis (-34.1%, p<0.001), hospitalization (-53.2%, p<0.001), and bone necrosis (-6.9%, p<0.001) (**Table 4,**

Figure 1). The median number of vaso-occlusive crisis per year was significantly lower on- compared with pre-hydroxyurea therapy (median: 3, range: 0-9 vs. median: 1, range: 0-9; p<0.001). Similarly, the median number of hospitalization episodes per year was significantly lower on- compared with pre-hydroxyurea therapy (median: 2, range: 0-8 vs. median: 0, range: 0-4; p<0.001). The median number of acute chest syndrome episodes per year was also significantly lower on- compared with pre-hydroxyurea therapy (median: 0, range: 0-8 vs. median: 0, range: 0-2; p<0.001). The median of silent cerebral infarctions was significantly higher compared with that pre-hydroxyurea therapy (+42.4%, p<0.001); in particular 19% of patients with normal MRI before the treatment had cerebral infarcts during HU therapy, and 81% of patients with previously detected SCI on MRI had a progression of cerebrovascular disease with new SCI. In **Table 5**, incidence of SCI pre- and on-hydroxyurea according to the descent. The mean dosage of HU in patients with SCI was 16.4±5.2 mg/Kg. Since ability to identify new silent cerebral infarction was restricted to patients who had underwent magnetic resonance imaging and hence naturally creating an imbalance between the number of patients with data pre- and on-hydroxyurea; we carried out sensitivity analysis using only patients who had magnetic resonance imaging in 'both' periods (n=84). No significant increase of stroke was observe (+0.5%, p=0.572), while silent infarcts were similar in both groups under hydroxyurea treatment, considering that patient sample was smallest in African descendent group.

The remaining complications, such as leg ulcers or pulmonary hypertension were comparable in incidence pre- and on-hydroxyurea therapy (**Table 4, Figure 1**). In **Table 5** and **Figure 2**, we show the incidence of complications pre- and on-hydroxyurea according to descent, age, genotype, duration on hydroxyurea .

The observed benefit in decreased incidence of acute chest syndrome, vaso-occlusive crisis, and hospitalization was observed universally across all subgroups. The benefit in decrease incidence of bone necrosis was primarily seen in adults (≥18 years), the βS/βS and βS/β^+ genotypes, patients on higher hydroxyurea dose (≥15 mg/kg/day) and not necessarily treated for long duration (<10 years); it was observed however in both Caucasian and African origins. The increased incidence in silent cerebral infarction was significant in adults, patients from Caucasian origin, and patients with βS/β^0 and βS/β^+ genotypes. It was also significant irrespective of dose, or duration of therapy (**Table 5** and **Figure 2**).

Safety

Adverse events as reported by treating clinicians were documented in 31/567 (5.5%) of patients. These included cytopenia (n=21), rash (n=1), melanonychia (n=1), nausea and poor appetite (n=1), allergic reaction (n=1), skin ulcers (n=1), and thrombosis (n=1) [1 patient's description was missing]. A total of 14/581 of patients (**Table 6)** developed cancer during hydroxyurea therapy (average age at cancer development 46.3 ± 13.1 years, median duration of hydroxyurea 10 years), corresponding to a tumor rate of 348 cases per 100,000 people versus the tumor rate of 385.1 cases per 100,000 people for Italian population reported by the International Agency for Research on Cancer (World Health Organization) (http://eco.iarc.fr/EUCAN/Cancer.aspx). All three patients who developed hepatocellular carcinoma had chronic hepatitis C infection, while one of the two patients developing lung cancer was a longtime heavy smoker. A total of 14 patients died during hydroxyurea therapy (all were ≥18 years, median duration on hydroxyurea was 10 years) (**Table 6**). A survival curve was constructed comparing

survival HU treated patients to a randomly selected group of 79 SCD patients (18 deaths, 22.8%) who had never received hydroxyurea, with follow up from birth until 31 December 2015, death, or loss to follow up (**Figure 3**). Because the two groups were not comparable for age ($p<0.001$) and in order to prevent the age-dependence of clinical complications [23] the comparison was performed with the subset of treated patients (n= 214) whose age was in the range between 25th and 75th percentile of the age of no treated subjects (i.e. 32 - 52.5 years). The data of these comparable groups ($p=0.86$) were used in the Cox model: the hazard ratio for death in patients who had used hydroxyurea during their disease course compared to those who did not was 0.22 (95% CI, 0.08-0.6, $p<0.0028$).

Regarding fertility, before HU administration only 30 men chose to store sperm samples. During the treatment sperm analysis showed azo/oligospermia in 3 patients.

Forty-three women were reported to be pregnant during hydroxyurea therapy (30 patients had one pregnancy, 11 had two pregnancies, and 2 had three pregnancies). Abortion in the first trimester of pregnancy was reported in 6 of the 58 pregnancies, while the remaining pregnancies continued through delivery with live births.

Complications during pregnancy were reported in seven of the 43 patients. All mothers discontinued hydroxyurea treatment during their gestation as soon as the pregnancy test was positive and were switched to either packed red blood cell transfusion or an exchange regimen. Hydroxyurea was only resumed when their lactation period was completed. The incidence of infertility treatment was 20% in Italian population.

DISCUSSION

This is the first large multicenter study in Southern European country, involving a large number of patients with different descent (Caucasian and Africans), referring to national comprehensive centers for hemoglobinopathies.

Our data indicate that hydroxyurea therapy lowers the incidence and annual frequency of acute chest syndrome, painful vaso-occlusive crisis, and hospitalization through a large cohort of SCD patients with either Caucasian or African descent.

Although established as a valuable therapeutic agent, in our study only 39.8 % of patients with SCD were treated with hydroxyurea. Other research suggests that hydroxyurea is underutilized in actual clinical practice for patients with SCD [23,24,25].

Such benefits were observed across the spectrum of patient profiles, irrespective of age, origin, genotype, and the dose or duration of therapy. These data confirm previous findings from clinical trials and long-term follow-up [7-13], and further shed light on the role of hydroxyurea in real-life management of patients with SCD [7-13].

Our data reflected a hydroxyurea treatment initiation period which goes earlier than recent studies [26,27] , and thus may represent a more conservative approach to management restricted to severe or older patients, thus explaining why no more than half of the population had received the drug. Our cohort is also characterized by a large proportion of immigrants from endemic areas for SCD, who were able to start hydroxyurea following referral to SCD centers at a later stage in their disease. Nonetheless, our results indicating benefit in such a patient admixture further support the wider use of hydroxyurea [2].

In our cohort, the incidence of stroke on hydroxyurea therapy was comparable to that pre-hydroxyurea, but new silent cerebral infarction was detected during HU

treatment. The onset was more frequent in those who had previous SCI, in agreement with previous reports in different SCD population [28,30]. This is of interest because in our study, owing to the retrospective nature of our study and lack of TCD or magnetic resonance angiography data, it is difficult to realize the cerebrovascular profile of evaluated patients and offer a robust conclusion regarding stroke prevention, although the lack of increase in incidence should be reassuring. However, our observation further raises a call for addressing the current knowledge gap on the role of hydroxyurea in prevention of silent cerebral infarction especially that data from smaller reports illustrate absence of hydroxyurea's role in this setting [29]. Moreover, through data stratification we were able to identify the subgroups at such increased risk of silent cerebral infarction and these included adult patients and those with sickle β-thalassemia which were either excluded or not adequately represented in recent trials [26-28]. The increased incidence of silent cerebral infarction with advancing age has been previously suggested [31]. The effect of co-inheritance of β-thalassemia, however, merits further evaluation.

We did not identify new types or higher incidence of adverse events on hydroxyurea therapy from those commonly published in the literature [13]. Considering that the majority of our cohort are adults, the observed rare cases of malignancy from various types are commensurate with what is observed in a surviving adult population [32]. To date, there is also no established negative impact of hydroxyurea therapy upon fertility and no clinical evidence of teratogenicity, although reports of pregnancy experience on hydroxyurea are limited [13]. Although our study cannot clearly confirm absence of risks, it adds to those limited experiences and further data in this direction is needed.

Our study does not come without limitations. The retrospective nature of data imposes missing values that could alter exact estimates of incidence rates, although there is no reason to believe that such shortcoming was dominant in the pre- or on-hydroxyurea periods. Moreover, several pathophysiologic markers and genetic factors that can alter the disease expression could not be evaluated or remain unknown, such data could have better characterized the patient profiles and the observed benefits or lack of in our cohort, some of which would have been altered within the natural course and regression to the mean. Our study did not evaluate compliance to therapy which could have implications on treatment effects. Lastly, certain peculiarities about the data analyzed herein, such as late onset of hydroxyurea start for some patients, indication often limited to what would be considered today severe cases, unbalanced exposure period before and on hydroxyurea, and potential under-dosing in some patient may not allow direct generalizability of results to other current patient populations.

Our real-life experience not only represents the first published nation-wide experience from Europe but further confirms the broad benefit and safety of a widely available therapeutic option for the prevention of acute chest syndrome, vaso-occlusive crisis, bone necrosis, and hospitalization in SCD, yet raises questions on its role in other disease manifestations. It also outlines some interesting clinical benefits, applicable to both children and adults, which are significant even with lower dosage than recommended and in subgroups which have been previously only scarcely been described in small studies like βS/β [31].

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AUTHORS' CONTRIBUTIONS

All authors participated in study concept and design, data collection and assembly, data analysis and interpretation, manuscript drafting, and critical revision for intellectual content.

DISCLOSURE OF CONFLICTS OF INTEREST

All authors have approved the final article. The authors declare no competing financial interests.

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Table 1. Patients' characteristics (n=628).

Parameter	Value
Age in years, mean ± SD, median (range)	24.5 ± 15, 24.0 (range: 1.0-67.0)
Age category in years, n/N (%)	
<18	209/554 (32.7)
≥18	345/554 (67.3)
Male, n/N (%)	320/622 (51.4)
Origin, n/N (%)	
Caucasian*	400/621 (64,4)
Africa**	221/621 (35.6)
Genotype, n/N (%)	
βS/βS	277/594 (46.6)
β^0/βS	167/594 (28.1)
β^+/βS	131/594 (22.1)
Other	19/594 (3.2)

SD, standard deviation.
*Caucasian: Albania, Saudi Arabia, India, Italy, Marocco, Tunisia
**African: Angola, Benin, Brasile, Burkina , Burkina Faso, Burundi, Camerun, Cina-Portorico, Congo, Costa D'Avorio, Egypt, Ghana, Guinea, Nigeria, Panama, Dominican Republic, Republic of Congo, Santo Domingo, Senegal, Sierra Leone, Togo, Uganda, USA

Table 2. Laboratory parameters pre- and on-hydroxyurea therapy.

Parameter	Pre-Hydroxyurea	On-Hydroxyurea	p-value
Total Hb (g/dl)	9.2 ± 1.6	9.7 ± 1.5	<0.001
HbF (%)	9.0 ± 8.0	17.0 ± 10.5	<0.001
HbS (%)	64.4 ± 14.2	57.1 ± 14.0	0.019
MCV (fL)	77.5 ± 9.9	91.1 ± 13.2	<0.001
WBC (x10^9/L)	12.1 ± 7.0	9.8 ± 13.5	0.001
Reticulocytes (%)	9.9 ± 10.1	8.6 ± 11.1	0.180
Platelets (x10^9/L)	386.1 ± 179.1	341.3 ± 174.0	<0.001
LDH (IU/L)	852.5 ± 954.2	691.2 ± 341.8	<0.001
Total bilirubin (mg/dL)	6.6 ± 14.9	6.2 ± 14.9	0.212
Direct bilirubin (mg/dL)	1.3 ± 2.4	1.3 ± 2.7	0.382
AST (IU/L)	43.6 ± 23.3	36.7 ± 24.1	<0.001
ALT (IU/L)	32.0 ± 25.3	34.2 ± 20.1	0.110

Data presented as mean ± standard deviation.
Hb, hemoglobin; MCV, mean corpuscular volume; WBC, white blood counts; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Table 3. Laboratory parameters by genotype, pre- and on-hydroxyurea therapy**.**

Parameter	Pre-Hydroxyurea			On-Hydroxyurea			p-value		
	βS/βS	βS/β0	βS/β+	βS/βS	βS/β0	βS/β+	βS/βS	βS/β0	βS/β+
Total Hb (g/dl)	8.5 ± 1.3	9.1 ± 1.2	10.5 ± 1.7	9.4 ± 1.4	9.5 ± 1.2	10.4 ± 1.5	<0.001	0.008	0.6
HbF (%)	9 ± 7	10 ± 7	6 ± 5	15 ± 9	21 ± 12	15 ± 11	<0.001	<0.001	<0.001
HbS (%)	61 ± 17	67 ± 14	66± 10	60 ± 14	54 ± 9	56 ± 12	0,34	0,004	0.004
MCV (fL)	83 ± 10	73 ± 6	73 ± 7	95 ± 13	87 ± 11	86 ± 11	<0.001	<0.001	<0.001
WBC (x10^9/L)	13 ± 8	12 ± 5	10± 6	10 ± 4	11 ± 23	8 ± 4	<0.001	0.7	<0.001
Reticulocytes (%)	14 ± 12	8 ± 5	9 ± 11	8 ± 11	7 ± 8	10 ± 15	0.013	0.7	0.8
Platelets (x10^9/L)	402 ± 163	404 ± 188	358 ± 227	360 ± 167	355 ± 167	320 ± 181	0.003	<0.001	0.1

Data presented as mean ± standard deviation.
Hb, hemoglobin; MCV, mean corpuscular volume; WBC, white blood counts

Table 4. Incidence of new complications pre- and on-hydroxyurea therapy.

Complication	Pre-Hydroxyurea	On-Hydroxyurea	Incidence difference	p-value
Stroke	17/620 (2.7)	20/622 (3.2)	+0.5%	0.572
Silent cerebral infraction	32/189 (16.9)	51/86 (59.3)	+42.4%	<0.001
Acute chest syndrome	227/556 (40.8)	69/598 (11.5)	-29.3%	<0.001
Vaso-occlusive crisis	501/555 (90.3)	336/598 (56.2)	-34.1%	<0.001
Hospitalization	343/433 (79.2)	159/612 (26.0)	-53.2%	<0.001
Pulmonary hypertension	14/484 (2.9)	9/353 (2.5)	-0.4%	0.210
Leg ulcers	28/498 (5.6)	28/619 (4.5)	-1.1%	1.000
Bone necrosis	103/417 (24.7)	61/343 (17.8)	-6.9%	<0.001
Chronic renal disease	17/474 (3.6)	13/320 (2.1)	-1.5%	0.774

All data presented as n/N (%).

Table 5. Incidence of complications pre- and on-hydroxyurea (HU) according to the descent.

Complication	Caucasian			African		
	pre-HU	on-HU	p-value	pre-HU	on-HU	p-value
Stroke	15/400 (3.8%)	20/396 (5.1%)	-	2/220 (0.9%)	0/220 (0%)	-
Silent cerebral infarction	26/147 (17.7%)	49/83 (59%)	<0.001	6/42 (14.3%)	2/3 (66.7%)	<0.001
Acute chest syndrome	146/357 (40.9%)	56/379 (14.8%)	<0.001	81/199 (40.7%)	13/218 (6.0%)	<0.001
Vaso-occlusive crisis	330/346 (95.4%)	230/392 (58.7%)	<0.001	171/208 (82.2%)	107/205 (52.2%)	<0.001
Hospitalization	243/312 (77.9%)	106/392 (27.0%)	<0.001	99/120 (82.5%)	53/219 (24.2%)	<0.001
Pulmonary hypertension	14/335 (4.2%)	8/314 (2.5%)	-	0/149 0%	1/38 2.6%	-
Leg ulcers	27/348 (7.8%)	26/399 (6.5%)	-	1/150 (0.7%)	2/219 (0.9%)	-
Bone Necrosis	85/336 (25.3%)	60/304 (19.7%)	<0.05	18/81 (22.2%)	1/37 (2.7%)	<0.05
Chronic Kidney disease	16/329 (4.9%)	12/281 (4.3%)	-	1/145 (0.7%)	0/37 (0%)	-

Table 6. Causes of death and cancer cases during hydroxyurea therapy.

Cause of death	**n**
Acute chest syndrome	4
Lung cancer	2
Pulmonary embolism	1
Hemorrhagic stroke	1
Live failure	1
Parvovirus infection in hepatic failure	1
Hepatocellular carcinoma	1
cardiac failure	1
Pulmonary hypertension	1
Infection	1
Cancer type	**n**
Hepatocellular	3
Breast	3
Lung	2
Nasopharyngeal	1
Laryngeal	1
Colon	1
Thyroid	1
Adrenal	1
Lymphoproliferative	1

FIGURE LEGENDS

Figure 1. Incidence of complications pre- and on-hydroxyurea. A significant reduction in the incidence ($p<0.001$) was found in complications: acute chest syndrome (-29.3%), vaso-occlusive crisis (-34.1%), hospitalization (-53.2%), bone necrosis (-6.9%); an increase was found in silent cerebral infarction ($p<0.001$, +42.3%).

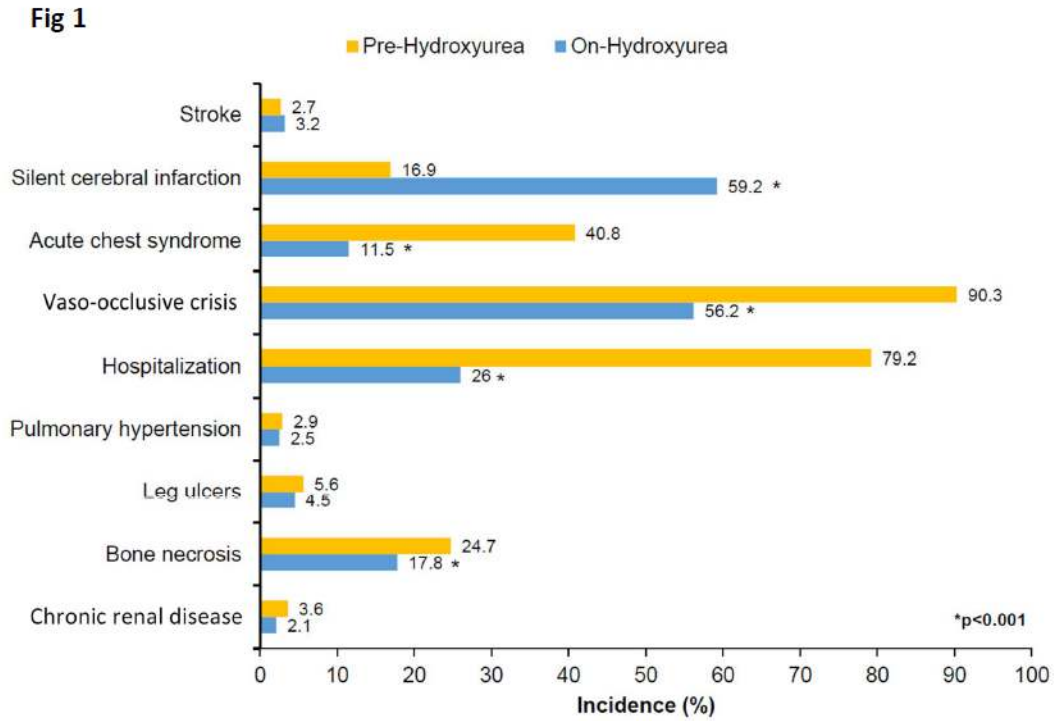
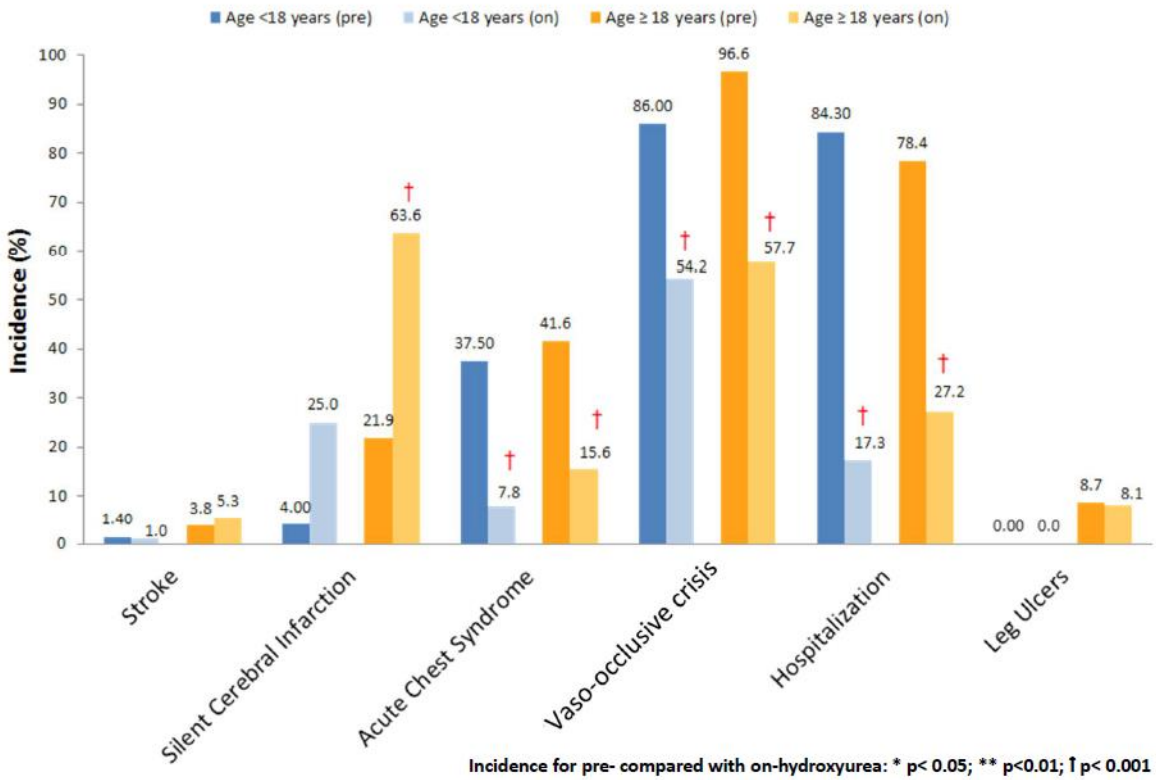


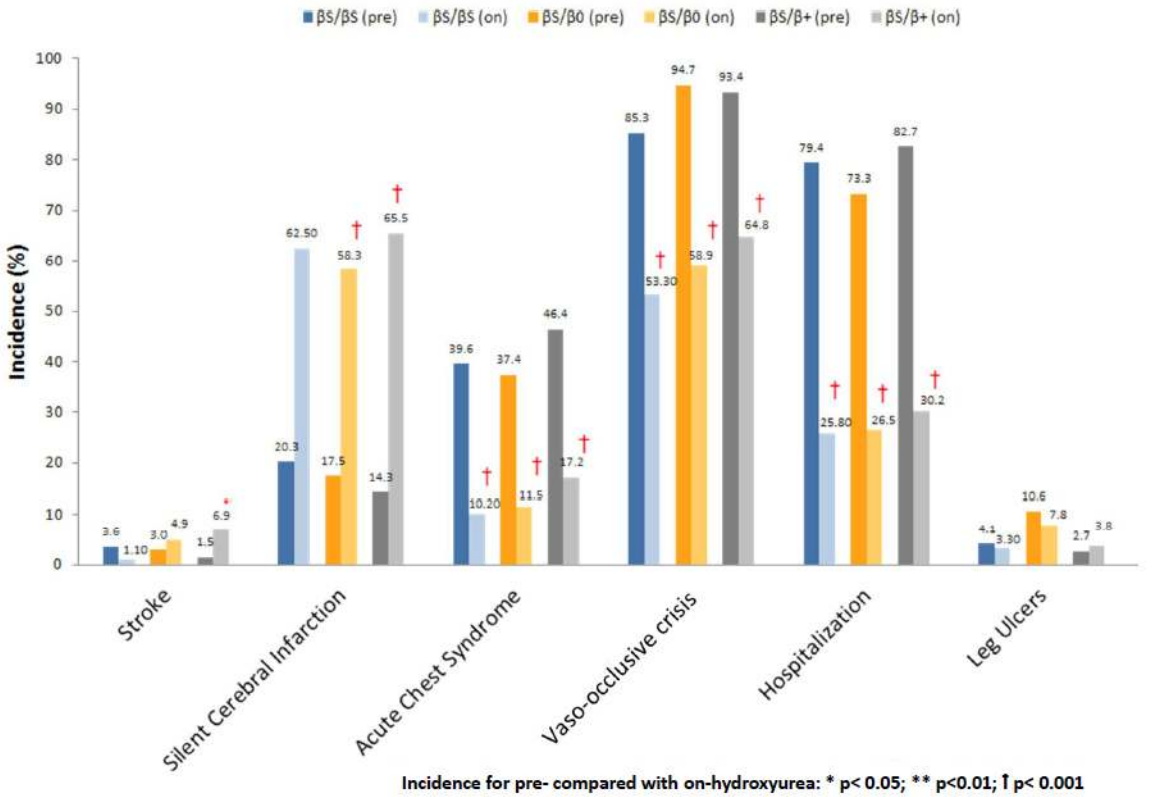
Figure 2. (A) Incidence of complications pre- and on-hydroxyurea according to the age of patients (i.e. patients with age ≤ 18 years and ≥ 18 years): a significant decrease ($p<0.001$) was found in acute chest syndrome (-29,7% and -26%), vaso-occlusive crisis (-31.8% and -38.9%) and hospitalization (-67% and -51.2%); an increase was found in silent cerebral infarction (+41.7%, $p<0.001$) of adult patients. **(B)** Incidence of complications pre- and on-hydroxyurea according to the genotypes βS/βS, βS/β0 and βS/β^+: a significant decrease ($p<0.001$) of the incidence was found in all genotype for acute-chest syndrome, vaso occlusive crisis and hospitalization, a significant increase ($p<0.001$) was found for silent cerebral infarction for groups βS/β0 and βS/β^+, an increase in stroke ($p<0.05$) for group βS/β^+. **(C)** Incidence of complications pre- and on-hydroxyurea according to the hydroxyurea (HU) duration (i.e. < 10 years and ≥ 10 years): a decrease ($p<0.001$) of the incidence was found for acute-chest syndrome, vaso-occlusive crisis and hospitalization, an increase was found in silent cerebral infarction ($p<0.001$) for both HU durations; a decrease ($p<0.05$) was found in necrosis for HU duration < 10 years.

Fig 2A



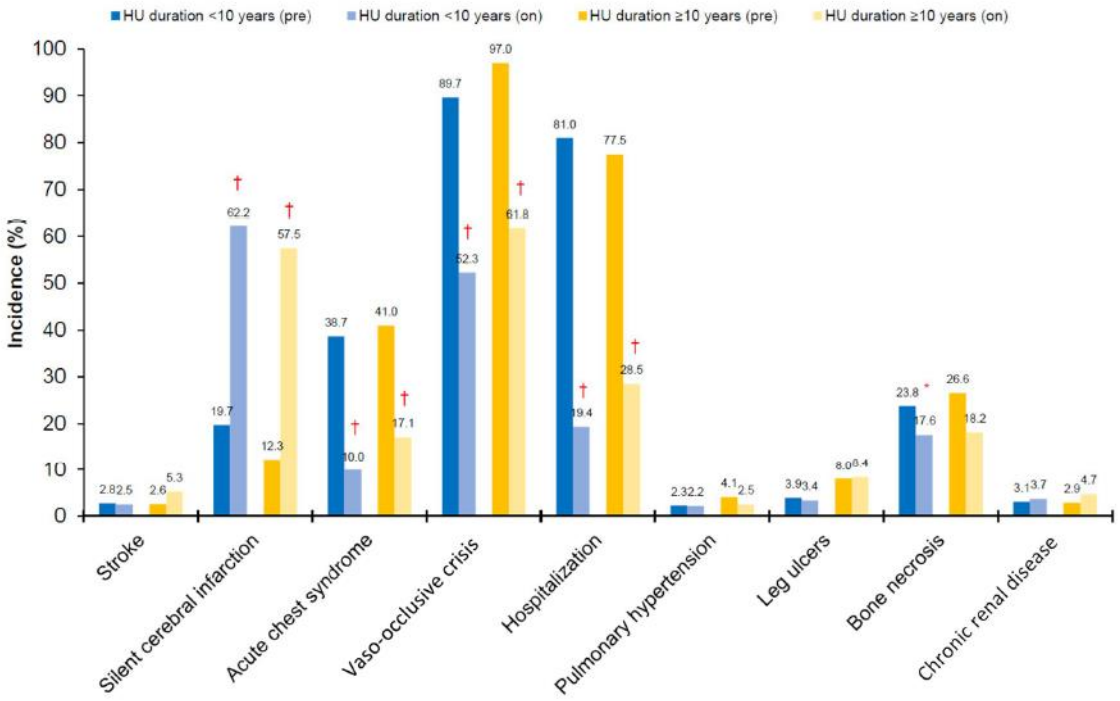
Incidence for pre- compared with on-hydroxyurea: * p< 0.05; ** p<0.01; † p< 0.001

Fig 2B



Incidence for pre- compared with on-hydroxyurea: * p< 0.05; ** p<0.01; † p< 0.001

Fig 2C



Incidence for pre- compared with on-hydroxyurea: * p< 0.05; ** p<0.01; † p< 0.001

Figure 3. Kaplan Meier curves shows the survival for patients treated with hydroxyurea (n=214) and no treated with hydroxyurea (n=79). The two survival curves are significantly different from each other (p=0.0012): patients receiving hydroxyurea have significantly better survival in comparison to those without.

Fig 3