The effect of prolonged administration of hydroxyurea on morbidity and mortality in adult patients with sickle cell syndromes: results of a 17-year, single-center trial (LaSHS)

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The aim of this prospective study was to evaluate the long-term efficacy and safety of hydroxyurea (HU) in patients with sickle cell disease (SCD). Thirty-four patients with sickle cell anemia (hemoglobin S [HbS]/HbS), 131 with HbS/β^0-thal, and 165 with HbS/β^+-thal participated in this trial. HU was administered to 131 patients, whereas 199 patients were conventionally treated. The median follow-up period was 8 years for HU patients and 5 years for non-HU patients. HU produced a dramatic reduction in the frequency of severe painful crises, transfusion requirements, hospital admissions, and incidence of acute chest syndrome. The probability of 10-year survival was 86% and 65% for HU and non-HU patients, respectively ($P = .001$), although HU patients had more severe forms of SCD. The 10-year probability of survival for HbS/HbS, HbS/β^0-thal, and HbS/IVSI-110 patients was 100%, 87%, and 82%, respectively, for HU patients and 10%, 54%, and 66%, for non-HU patients. The multivariate analysis showed that fetal hemoglobin values at baseline and percentage change of lactate dehydrogenase between baseline and 6 months were independently predicted for survival in the HU group. These results highlight the beneficial effect of HU, which seems to modify the natural history of SCD and raise the issue of expanding its use in all SCD patients. (Blood. 2010;115:2354-2363)

Introduction

Sickle cell disease (SCD) comprises a group of hereditary disorders characterized by sickling of erythrocytes when they are deoxygenated. Patients with SCD have both acute and chronic episodic pain and reduced quality and quantity of life.[1-3] SCD encompasses several different genotypes that include sickle cell anemia (hemoglobin S [HbS]/HbS) and compound heterozygotes of HbS and β-thalassemia (HbS/β-thal) or with other types of hemoglobinopathies. The underling molecular defect of the β-gene can produce some percentage of β chains (the IVS1-110 mutation may produce hemoglobin A [HbA] up to 10% of HbA^+, whereas the IVS1-6 mutation may produce 10%-20% of HbA, HbA^{++}) or produce no β chains (HbA^0). The presence of HbA or hemoglobin F (HbF) within sickle erythrocytes decreases sickling and decelerates the polymerization process.[4,5]

Hydroxyurea (HU) is a chemotherapeutic agent that had been used for decades to reduce abnormally high blood cell counts in patients with myeloproliferative disorders. It inhibits the enzyme ribonucleotide reductase and thus causes cell-cycle arrest and allows γ-globin genes to be more actively expressed. By killing cycling cells, HU changes the kinetics of erythroid proliferation, forcing more F cells to be produced from primitive progenitors and directly stimulating HbF production.[6] The efficacy of HU in the treatment of SCD is generally attributed to its ability to increase HbF.[7-9] Furthermore, HU reduces the number of white cells, platelets, and reticulocyte counts that further contribute to vascular injury and reduces the adhesiveness of sickled red cells to the endothelium,[3,10,11] whereas nitric oxide seems to participate in HU's beneficial effect on inducing HbF levels in erythroid progenitors.[12,13]

HU is now considered as the main pharmacologic agent capable of preventing the complications of SCD and improving the quality of life of SCD patients, and it is approved by both the Food and Drug Administration (in 1999) and European Medicines Agency (in 2008). Despite the available data, the mechanism of HU action in SCD has not been fully clarified, and whether HU can prevent the severe chronic complications or modify the mortality rate of these patients remains an unresolved question. In an attempt to address this issue, we designed a prospective trial in which HU was given for a long period in adult patients with SCD who were followed in a single center, that is, The Laikon Study of Hydroxyurea in Sickle Cell Syndromes (LaSHS study). The primary end point of the LaSHS study was the efficacy and safety of HU in SCD and especially its effect on SCD complications, such as painful crises, need for transfusion, acute chest syndrome, and strokes. A secondary end point was the overall survival (OS) of the patients who received HU compared with the OS of the patients who did not receive HU and who were followed in the same institute.

Methods

Study design

The LaSHS prospective phase 2 study was conducted in the Thalassemia Center of Laikon General Hospital in Athens, Greece, to evaluate the efficacy and safety of HU in SCD. According to protocol, the survival and

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the incidence of chronic complications of patients who received HU was compared with the respective values of all other SCD patients who were treated with conventional therapy, were followed-up in the same center, and agreed to participate in this study.

The study was started in 1991 and was supported by the Greek Ministry of Health after approval of the Greek National Drug Organization (Ref No. A2γ/667/24-2-91 and Y3α/1210/21-2-94). Informed consent was obtained from all patients before inclusion in the study in accordance with the Declaration of Helsinki. Before the administration of HU, patients who fulfilled the criteria to receive HU consented regarding (1) the potential long-term toxicity of HU, especially regarding the occurrence of secondary leukemia or cancer (which was referred as unknown in SCD patients but low in other diseases treated with HU); (2) fertility, with the suggestion of frozen sperm storage for male patients, whereas female patients were encouraged to use contraception; all patients (both male and female) had to discontinue their treatment 4 months before procreation because HU might affect fertility; and (3) frequent visits for clinical and laboratory examinations every 15 days during the first 2 months of therapy and then every 4 to 8 weeks. Patients who continued on conventional therapy allowed us to use all their laboratory and clinical data for comparisons with patients who received HU. These patients could receive HU at any time when they fulfilled the inclusion criteria for HU administration.

The initial duration of the study was 2 years. After the very good results observed in our patients at 2 years, we extended the trial for 5 more years and after Food and Drug Administration approval of HU for SCD in 1998, we further extended the study till the drug would be available in Greece (European Medicines Agency approval) to give the opportunity to more patients who are eligible to receive the drug.

Inclusion criteria

The inclusion criteria for entrance into LaSHS study included age greater than 16 years, 3 or more painful sickle cell crises during the preceding year that needed hospitalization or emergency room visits; the presence of jaundice at presentation or complications of SCD, such as stroke and acute chest syndrome during the last 5 years; white blood cell counts of 3×10^9/L or greater with neutrophil counts of 1.5×10^9/L or greater; and platelet counts 100×10^9/L or greater.

Exclusion criteria

The exclusion criteria included fewer than 2 painful sickle cell crises during the preceding year that needed hospitalization or emergency room visits; the presence of end-stage renal failure (patients on dialysis); pregnancy in female patients; and no patient agreement on regular visits for blood tests and mental inability to sign the informed consent.

Treatment schedule

The starting dose of HU was 20 mg/kg per day in a single oral dose. As the result of toxicity or lack of effectiveness, dose modifications were required. When neutrophil counts were less than 1.5×10^9/L and platelet counts were less than 100×10^9/L, administration of HU was discontinued till recovery. Then the patient restarted HU treatment at a dose of 15 mg/kg per day, which increased gradually to the dose before toxicity within 1 month. If the patient showed no recovery after 2 months of HU discontinuation, they were excluded from the study. If there was no response to HU therapy after 3 months with the starting HU dose, the dose of HU was gradually increased to 35 mg/kg per day (increment of 5-10 mg/month). If there was no response after 6 months in that dose, the patient was withdrawn from the study.

HU could be discontinued for 4 months before procreation for both male and female patients. If a pregnancy was confirmed within this period of time, the male patients restarted HU, whereas female patients did not receive HU until delivery and then started again. However, the period of discontinuation till pregnancy could not exceed 6 months.

Pretreatment, efficacy, and safety assessments

Pretreatment evaluations consisted of patients' history, physical examinations, standard hematology and biochemistry, HbF measurements, and underlying molecular mutations, as previously described.[14] A negative pregnancy test was required for all women of childbearing potential. Before starting treatment, all patients were asked to record by memory on an arbitrary scale all painful events that occurred 12 months before entering the clinical trial.[14,15] Patients were followed every 2 weeks for the first 2 months after starting HU and then every 4 to 8 weeks for thorough laboratory follow-up, including full blood count, reticulocyte counts, HbF measurement, and standard serum biochemistry. Patients who did not receive HU were also in a regular follow-up every 4 to 8 weeks, having similar thorough clinical and laboratory checking from the same treating physicians.

Evaluation of response was monitored every 4 to 8 weeks. Response was defined as 2-fold increase of HbF levels after 6 months of HU treatment compared with baseline HbF values and improvement of clinical status with reduction of painful crisis for at least 50% within 12 months of HU administration.

All patients who received at least 1 month of HU therapy were eligible for assessment of toxicity. Toxicity was graded according to the National Cancer Institute Common Terminology Criteria Version 2.0.

Statistical analysis

Data were analyzed as of July 2008. The Mann-Whitney *U* test was used to evaluate differences between patients who received or did not receive HU. The nonparametric Kolmogorov-Smirnov test, the Wilcoxon nonparametric test, and analysis of variance (with post-hoc Bonferroni analysis) were used to evaluate differences for laboratory parameters at different time points. Nonparametric Fiedman and Wilcoxon tests as well as the McNemar test were used to evaluate differences between clinical parameters at different time points. The Spearman nonparametric correlation test determined the correlations between evaluated parameters. The Kaplan-Meier method was used to estimate OS probabilities, with differences compared by the 2-sided log-rank test to identify potential prognostic factors. We performed univariate analyses using the Fisher exact test for categorical factors and logistic regression for continuous factors. Survival was calculated from the date of initiation of HU treatment until the date of death, by any cause, or the date of last contact for patients who received HU. For patients who did not receive HU, survival was calculated from the date of initiation of HU therapy of the first patient who entered into HU study. Patients who were lost to follow-up were censored at the date of last contact. Variables found to be statistically significant at P less than .1 were entered into a Cox proportional hazards regression analysis that used a backward stepping procedure to identify the more important factors with independent significance. All P values were 2-sided, and confidence intervals refer to 95% boundaries.

Results

Patients

Since 1991, 330 patients (136 men/194 women; median age, 42 years; range, 20-76 years) were followed-up in the Thalassemia Center of Laikon General Hospital in Athens, Greece. Thirty-four patients had sickle cell anemia (HbS/HbS), and 296 were compound heterozygotes for HbS and β-thalassemia: 131 patients with HbS/β^0-thal and 165 patients with HbS/β^+-thal. The underlying molecular mutations of β-gene were IVSI-110 (107 patients) and IVSI-6 (58 patients). One hundred thirty-one patients with severe disease burden received HU, whereas 199 patients, most of them with very good clinical status, were conventionally treated (eg, analgesics, hydration, oxygen). Follow-up data for up to 17 years were completed for 131 patients who received HU treatment. The median follow-up for HU patients was 8 years (range, 0.1-17 years), whereas the median follow-up for patients who did not receive was 5 years (range, 0.1-18 years). The difference of the follow-up between the 2 groups is explained by

Table 1. Main characteristics of the patients at baseline

	HU (n = 131)		Non-HU (n = 199)		
Baseline	**Median**	**SD**	**Median**	**SD**	***P***
Age, y	33.0	11.2	35.0	12.8	.506
Follow-up, y	8.0	4.7	5.0	6.0	.009
Blood units/y	0	5.86	0	2.83	.004
Hospital admissions/y	1.00	2.95	0	1.18	.000
Painful crises/y	6.00	6.49	1.00	3.81	< .001
Hb, g/dL					
HbS/β^0	8.90	1.34	9.20	1.34	.133
HbS/β^+	9.60	1.25	9.60	1.77	.407
HbS/HbS	8.70	1.51	8.90	2.00	.589
Leukocyte counts, $\times 10^9$/L					
HbS/β^0	11.98	6.73	9.70	6.16	.045
HbS/β^+	8.40	5.44	8.70	4.73	.109
HbS/HbS	11.48	2.90	12.50	2.86	.445
Platelet counts, $\times 10^9$/L					
HbS/β^0	332.0	215.0	280.0	155.9	.096
HbS/β^+	203.0	142.9	252.5	169.3	.027
HbS/HbS	463.5	154.5	382.5	131.9	.079
HbF, %					
HbS/β^0	7.80	5.96	6.70	8.02	.947
HbS/β^+	6.80	4.67	5.10	5.13	.380
HbS/HbS	5.75	3.79	4.00	2.91	.079
Reticulocytes, %					
HbS/β^0	10.0	7.4	8.0	7.7	.006
HbS/β^+	9.0	7.41	6.0	5.45	.017
HbS/HbS	12.0	6.5	10.0	4.9	.091
Bilirubin, mg/dL					
HbS/β^0	1.65	3.91	1.80	2.07	.410
HbS/β^+	1.82	2.26	1.73	1.25	.317
HbS/HbS	2.81	2.80	2.70	1.70	.858
LDH, U/L					
HbS/β^0	766.0	464.1	674.0	370.0	.025
HbS/β^+	698.0	362.3	717.0	300.6	.960
HbS/HbS	824.5	341.6	688.0	377.6	.022
MCV, fL					
HbS/β^0	71.6	4.4	72.9	3.8	.565
HbS/β^+	69.4	4.1	68.9	5.1	.628
HbS/HbS					
	n	**%**	**n**	**%**	
Sex					
Male	67	51.1	69	34.7	.002
Female	64	48.9	130	65.3	
Genotype					
HbS/β^0	63	48.1	64	33.7	< .001
HbS/IVS1-110	42	32.1	64	32.6	
HbS/IVS1-6	2	1.5	54	28.4	
HbS/HbS	24	18.3	10	5.3	
History of chest syndrome at baseline	8	6.1	8	4.0	.388
History of aseptic necrosis at baseline	16	12.2	17	8.5	.183
History of stroke at baseline	4	3.1	1	0.5	.063
Liver dysfunction at baseline	33	25.1	54	27.1	.312
Leg ulcer at baseline	3	2.2	5	2.5	.618

As "history of an event" at baseline, we define the presence of the event whenever before baseline.
Hb indicates hemoglobin, HU, hydroxyurea; LDH, lactate dehydrogenase; MCV, mean cell volume.

the greater number of referral patients who fulfilled the criteria for HU administration during the first 10 years of the study; this number was dramatically reduced during the last 3 years of the study, when the majority of patients who presented in our center had mild forms of the disease and did not fulfill the criteria for HU use (Table 1).

The main characteristics of all patients are depicted in Table 1. As expected by the inclusion criteria of the study, HU patients had a greater rate of painful crises per year, greater admission rate, greater transfusion requirements, greater reticulocytes counts, and greater lactate dehydrogenase (LDH) levels compared with patients who did not receive HU. In terms of clinical characteristics, HU

Table 2. Dramatic reduction of hospital admissions, painful crises, and transfusion requirements per year of the study in patients who received HU (data as of June 30, 2008)

Years receiving HU	No. of patients receiving HU	Hospital admissions per year, mean ± SD	Painful crises per year, mean ± SD	Transfusion requirements, mean ± SD of red-cell packs per year
Baseline	131	2.11 ± 2.96	7.34 ± 6.50	1.53 ± 5.92
1	126	0.03 ± 0.19	0.05 ± 0.22	0.22 ± 0.95
2	120	0.04 ± 0.21	0.04 ± 0.20	0.19 ± 0.87
3	109	0.04 ± 0.21	0.03 ± 0.18	0.08 ± 1.54
4	104	0.01 ± 0.13		0
5	97	0.04 ± 0.19	0.03 ± 0.15	0
6	86	0.02 ± 0.15	0	0
7	80	0.05 ± 0.20	0.02 ± 0.15	0.03 ± 0.19
8	76	0.05 ± 0.22	0.02 ± 0.16	0
9	67	0.07 ± 0.26	0.04 ± 0.20	0
10	61	0.05 ± 0.22	0	0
11	56	0.06 ± 0.15	0.01 ± 0.13	0.03 ± 0.18
12	48	0.06 ± 0.24	0.02 ± 0.14	0.04 ± 0.20
13	42	0.04 ± 0.21	0	0
14	33	0.06 ± 0.24	0.03 ± 0.17	0.06 ± 0.34
15	26	0.03 ± 0.19	0.03 ± 0.19	0
16	18	0.05 ± 0.23	0	0
17	12	0	0	0

HU indicates hydroxyurea.

group included a lower number of female patients and a lower number of patients with IVSI-110 and IVSI-6 mutations compared with those conventionally treated.

Safety data

All 131 patients were eligible for safety and toxicity evaluation. The mean dosage (±SD) of HU that was given in patients with SS and HbS/β^0-thal was 20.5 (± 4.2) mg/kg per day per study year, whereas the mean dosage of HU achieved in patients with HbS/β^+-thal was 17.5 (± 3.2) mg/kg per day per study year. Nine patients showed neutropenia; 2 of grade 1, 4 of grade 2, and 3 of grade 3. Furthermore, 8 patients developed thrombocytopenia; 3 of grade 1, 4 of grade 2, and 1 of grade 3. Three patients discontinued HU therapy as the result of repeated neutropenia or thrombocytopenia, but they reentered into the study after recovery. Two patients developed red cell aplasia after 10 and 5 years on HU treatment, respectively, but they both reentered into the study after 6 and 7 months, respectively, when they recovered from their crises, at a lower dose of HU (15 mg/kg per day) without any other complication. Two patients refused the frequent and regular visits with clinical and hematologic control, and they stopped the treatment after being in the trial for 3 and 5 years. One patient developed melanonychia, and 2 female patients developed grade 1 alopecia. No other side effects were observed in the study. There were no cases of myelodysplasia or cancer during the study period in both patients who received HU and in those who did not receive HU.

Regarding fertility, before HU administration 15 men (22.3%) chose to store sperm samples. The results of the sperm analysis showed that of those 15 men, 9 were found to be oligospermic, and 2 patients had azoospermia. While on study, 6 male and 5 female patients managed to carry out a normal delivery after discontinuation of therapy for 4 months (this was obligatory according to written consent), without any specific therapy or problems during pregnancy. Two of these 6 male patients continued HU through conception, whereas no female patient continued HU through the entire pregnancy. One male and 1 female patient had 2 children during 10 years of HU therapy. Two men procreated while receiving HU therapy, and their female partners delivered a healthy baby; 1 of the men procreated twice while on 12 years of HU therapy. This patient informed us about the healthy deliveries after the birth of his children in both cases, although he was very well informed, not only in the study entrance (when he had signed the written consent) but also every 3 visits (as this was the standard procedure during follow-up period). No babies born to patients who were under HU had birth defects or any other problems.

Efficacy data

On the basis of LaSHS study definition of response, all patients who received HU responded to therapy, and no patients was excluded from the analysis because of lack of response to HU. Thus, all these patients continued on treatment until the last follow-up (June 2008). The patients had a median of 5-fold increase of HbF (range, 2- to 11-fold increase) at 6 months. During the study period (April 1991 to June 2008), patients who received HU showed: (1) a dramatic reduction of the frequency of severe painful crises (mean ± SD: from 7.34 ± 6.5 episodes per year before HU to 0.025 ± 0.026 episodes per study year after HU; $P < .001$); (2) a significant reduction of transfusion requirements (mean number of administered packed red cell units: 1.5 ± 5.9/year before HU diminishing to almost zero during HU treatment; $P < .001$); (3) a significant reduction of hospital admissions from 2.11 (± 2.96)/year before HU to 0.041 (± 0.018) per study year after HU ($P < .001$; Table 2); and (4) a significant reduction of the incidence of chest syndrome (from 6.1% of patients before HU to 0.8% of patients during the HU period; $P = .016$).

HU administration produced a significant increase of total Hb and HbF at 6 and 12 months after HU initiation and at last follow-up along, with a significant reduction of leukocyte counts, platelet and reticulocyte counts, serum bilirubin, and LDH levels (Table 3). In contrast, patients who were conventionally treated demonstrated a significant reduction of Hb and platelet counts between baseline and last follow-up, whereas they did not show any significant changes in reticulocyte counts, serum bilirubin, and LDH levels at the same time period (Table 3; for non-HU patients, baseline values were considered the values when the LaSHS study was started).

Table 3. Changes of laboratory parameters in patients who received HU and in patients who were treated with conventional therapy

Parameter	Before HU treatment, median (range)	After 6 months, median (range)	After 12 months, median (range)	Last follow-up, median (range)
Patients on HU				
Hb, g/dL	9.2 (5.5-12.3)	9.8* (7.3-13.5)	9.6* (6.8-13.5)	9.5† (6.3-13.0)
MCV, fL	71.2 (63.4-99.7)	99.7* (77.8-133.6)	98.4* (79.7-126.8)	96.8* (79.8-127.2)
Leukocyte counts, $\times 10^9$/L	10.7 (3.0-33.8)	7.1* (3.0-21.8)	7.3* (3.1-20.9)	8.0* (2.9-39.6)
Neutrophil counts, $\times 10^9$/L	7.6 (2.3-24.1)	4.7* (2.0-16.3)	4.5* (1.5-14.8)	4.0* (1.4-22.9)
Platelet counts, $\times 10^9$/L	306 (100-900)	274* (72-864)	291* (82-815)	308† (31-872)
HbF, %	6.8 (1.0-27.4)	21.8* (1.0-49.0)	20.4* (3.0-49.0)	17.4* (0.8-38.3)
Reticulocyte, %	10 (1-40)	6* (1-23)	7* (1-35)	6* (1-18)
Bilirubin, mg/dL	1.9 (0.4-23.5)	1.5* (0.2-23.5)	1.5* (0.3-11.6)	1.4* (1-18)
LDH, U/L	742 (180-2180)	633* (175-1471)	643* (252-1904)	636* (186-2131)
Patients on conventional therapy				
Hb, g/dL	9.4 (5-14.6)	9.0* (5.9-14.2)	9.0* (5.5-12.8)	9.1‡ (5.5-13.6)
MCV, fL	71.4 (63.8-99.2)	70.8 (63.2-100.2)	70.6 (63.1-99.6)	71.1 (62.8-99.2)
Leukocyte counts, $\times 10^9$/L	9.1 (2.2-33.4)	9.1 (1.2-57.0)	8.3* (3.3-35.9)	9.3 (3.1-79.0)
Neutrophil counts, $\times 10^9$/L	5.4 (1.5-24.8)	5.8 (0.7-43.7)	5.5 (1.6-27.2)	5.6 (1.6-56.8)
Platelet counts, $\times 10^9$/L	269 (83-888)	325 (43-900)	258 (89-787)	231* (35-710)
HbF, %	5.3 (0.5-32.0)	5.0* (1.0-17.0)	5.0* (1.0-29.0)	4.9* (0.7-30.0)
Reticulocyte, %	7 (1-40)	8 (1-40)	8 (2-28)	7 (1-25)
Bilirubin, mg/dL	1.9 (0.3-11.0)	1.8 (0.3-23.5)	2.1 (0.8-11.1)	1.8 (0.4-38.0)
LDH, U/L	680 (180-2210)	700‡ (180-2180)	750‡ (310-1880)	721‡ (221-3064)

Hb indicates hemoglobin, HU, hydroxyurea; LDH, lactate dehydrogenase; and MCV, mean cell volume.
*$P < .001$ compared with baseline values.
†$P < .01$ compared with baseline values.
‡$P = .01$ compared with baseline values.

A beneficial effect of HU also was observed on chronic complications of SCD (Table 4). In terms of strokes, from April 1991 to June 2008, there were 5 cases in patients who received HU treatment, and 3 of them died as the result of intracerebral hemorrhage compared with 10 cases who presented and died as the result of stroke in the group of patients who did not receive HU (death rate caused by stroke 2.2% vs 5% for HU and non-HU patients, respectively). Similarly, the death rate of liver dysfunction in patients who were given HU was 0.7% compared with 5% on patients who were not given HU (Table 4). In our study, at baseline, 16 of 131 HU patients (12.2%) had a history of symptomatic avascular necrosis of the femoral neck (n = 14) or the shoulder (n = 2). All but 2 patients (who have not decided yet to undergo a surgery) had been successfully underwent a surgical replacement of the head of the femur with restitution of the joint. During HU therapy, only 1 patient presented accelerated avascular necrosis of the femoral neck 3 months after initiation of HU treatment.

In the group of patients who did not receive HU, at baseline, 17 (8.5%) had a history of avascular necrosis, and 6 more (3%) developed avascular necrosis of the femoral neck during follow-up period. Finally, in terms of leg ulcers, at baseline, 3 patients had chronic leg ulcers before the initiation of HU (2.3%). During HU administration these patients continued to present recurrences of the ulcers in a more aggressive form, but no new case was observed. Similarly in non-HU group, the prevalence of leg ulcers was 2.5% (5 patients of 199) with remissions and recurrences. All patients had a very good compliance as reflected by their answers during the clinical visits and the results of MCV; however, a more accurate way to estimate patients' adherence to medication (ie, special boxes of HU with electronic system, etc) was not used.

Table 4. Causes of death in HU and non-HU patients

Cause of death	HU patients (13/131 = 9.9%)	Non-HU patients (49/199 = 24.6%)
Liver dysfunction	1	10
Pulmonary hypertension	8	8
Stroke	3	10
Sudden death	3	5
Vasoocclusion crisis	1	6
Acute chest syndrome	1	5
Sepsis	1	1
Heart failure	2	2
Intervention	1	2

Causes of death were diagnosed contemporaneously by study staff in 45 of 62 patients who died in the center hospital. In all other patients the cause of death was diagnosed by the respective doctors of the hospitals where the death was happened; no autopsy was performed in these patients.
HU indicates hydroxyurea.

Survival data

The death rate in patients who received HU was significantly lower than that observed among patients who were conventionally treated (13 deaths in HU group vs 49 in non-HU group; 9.9% vs 24.6%, respectively; $P = .001$; Table 4). On an "intent-to-treat" basis, the probability of 10-year survival was 86% for the patients treated with HU, whereas that of the patients conventionally treated was only 65% ($P = .001$; Figure 1). The cause of death for each group of patients is depicted in Table 4. It is clear that patients who did not receive HU had more deaths as the result of strokes, vasoocclusive crises, liver dysfunction, and pulmonary hypertension.

Among patients who received HU and died during therapy, no patients had HbS/HbS, 7 (5.34%) had HbS/β^0-thal, 4 (3.05%) had the HbS/IVSI-110 mutation, and 2 (1.5%) had the HbS/IVSI-6 mutation. On the contrary, in patients who did not receive HU and died during follow-up period, 10 (5.02%)patients had HbS/HbS, 22 (11.05%) had HbS/β^0-thal, 14 (7.03%) had the HbS/IVSI-110 mutation, and 3 (1.5%) had the HbS/IVSI-6. The Kaplan-Meier analysis also confirmed that the beneficial effect of HU on survival was mainly in HbS/HbS and HbS/β^0-thal patients (Figure 2). In patients who received HU, the 10-year probability of survival for HbS/HbS, HbS/β^0-thal, and HbS/IVSI-110 patients was 100%, 87%, and 82%, respectively. In patients who did not receive HU,

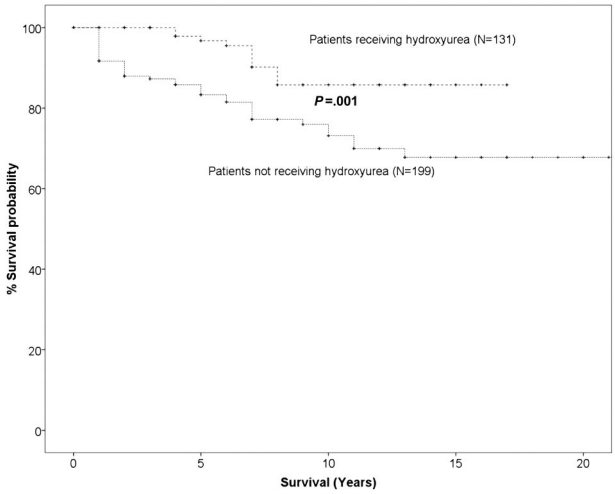

Figure 1. Probability of 10-year overall survival in sickle cell disease (SCD) patients who received hydroxyurea and in SCD patients who were conventionally treated: 86% versus 65% ($P = .001$).

the 10-year probability of survival for HbS/HbS, HbS/β^0-thal, and HbS/IVSI-110 patients was 10%, 54%, and 66%, respectively.

Univariate analysis showed that HbF at baseline at a cutoff value of 2%, reticulocytes counts at a cutoff value of 2%, and percent change of LDH between baseline and 6-month values as a continuous variable predicted for survival (Table 5). Patients who had HbF values of greater than 2% had a 10-year probability of survival of 89%, whereas all other patients had a 10-year probability of survival of 53% ($P = .001$; Figure 3). Furthermore, bilirubin as a continuous variable had a borderline significant predictive value for survival (Table 5). However, the multivariate analysis showed that only HbF and percent change of LDH between baseline and 6-month values were independently predicted for survival (Table 6).

We also performed an analysis to reveal predictive factors for survival in patients who did not receive HU. In this cohort of patients increased levels of Hb and low levels of bilirubin at baseline were independent prognostic factors for superior survival. Tables 7 and 8 depict the results of the univariate and multivariate analyses in this cohort of patients.

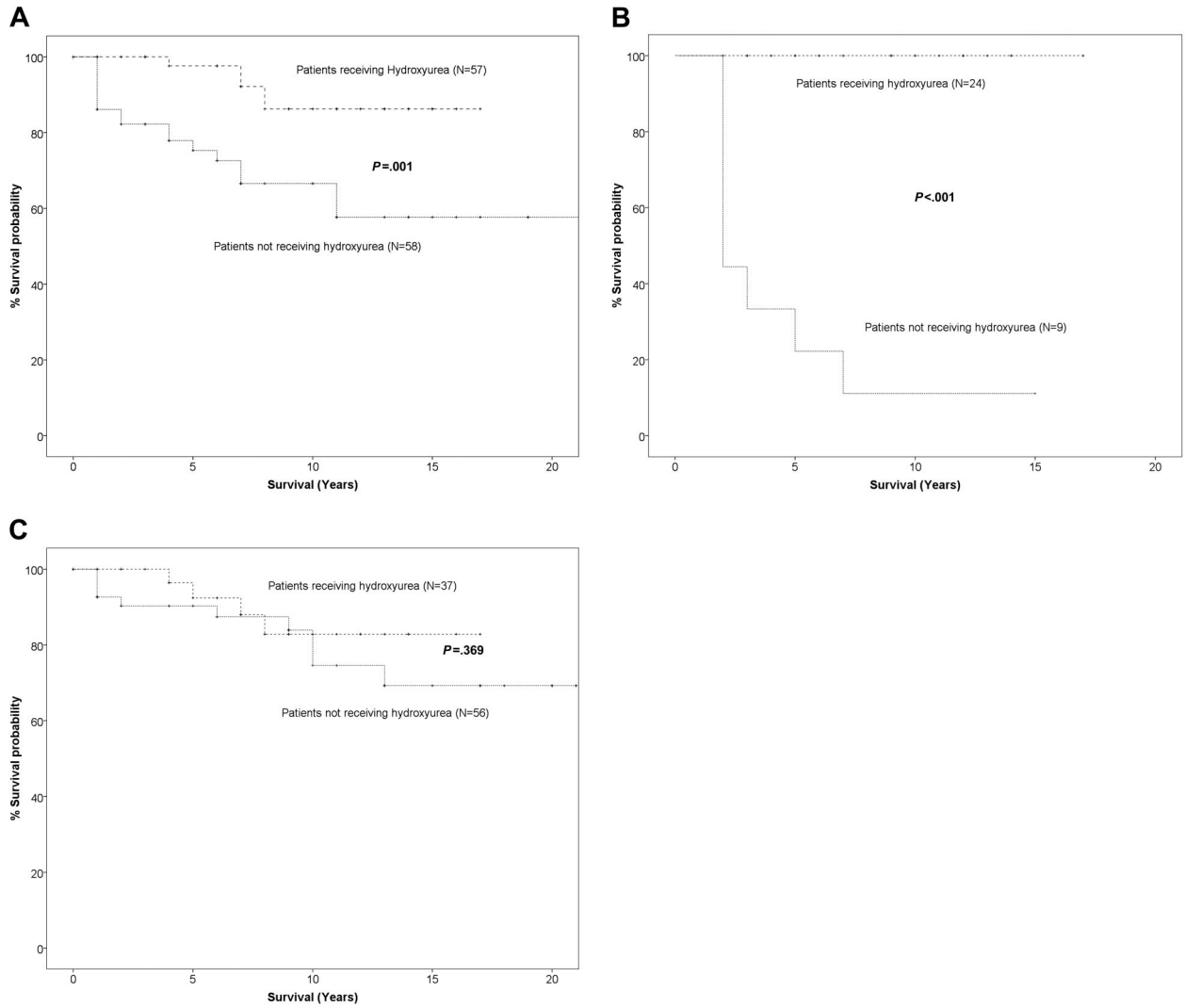

Figure 2. Probability of 10-year overall survival according to molecular subtypes of SCD. Patients with hemoglobin S (HbS)/β^0-thal who received hydroxyurea (HU) had a 87% probability of 10-year overall survival (OS) compared with 54% of non-HU patients ($P = .001$; A). The respective 10-year probability for HU patients with HbS/HbS and HbS/IVSI-110 was 100% (B), and 82% (C), whereas for non-HU patients these probabilities were 10% ($P < .001$; B) and 66% ($p = 0.369$; C), respectively.

Table 5. Factors studied in the univariate analysis for predicting survival in patients who received HU

Variable	Odds ratio (*P*)	95% CI
Age, y (continuous variable)	0.992 (.792)	0.935-1.053
Hemoglobin dichotomous variable (> 9 g/dL), continuous variable	0.593 (.374)	0.187-1.876
	0.883(.528)	0.601-1.299
HbF dichotomous variable (> 2%), continuous variable	0.243 (.038)	0.064-0.922
	0.945(.441)	0.817-1.092
White blood cell counts dichotomous variable (> 8×10^9/L), continuous variable	1.465 (.567)	0.396-5.419
	1.000(.496)	1.000-1.000
Platelet counts dichotomous variable (> 100×10^9/L), continuous variable	0.786 (.683)	0.247-2.497
	1.000(.563)	1.000-1.000
Reticulocyte counts dichotomous variable (< 2%), continuous variable	0.106 (.005)	0.022-0.502
	1.014(.723)	0.940-1.093
Bilirubin dichotomous variable (> 1.2 mg/dL), continuous variable	0.931 (.916)	0.250-3.472
	1.094(.056)	0.998-1.200
LDH dichotomous variable (> 800 U/L), continuous variable	0.892 (.723)	0.267-2.150
	0.999(.425)	0.997-1.001
Percent change of Hb between baseline and 6th month (continuous variable)	0.997 (.873)	0.959-1.036
Percent change of WBC between baseline and 6th month (continuous variable)	0.992 (.448)	0.972-1.013
Percent change of PLT between baseline and 6th month (continuous variable)	0.993 (.399)	0.977-1.009
Percent change of HbF between baseline and 6th month (continuous variable)	1.001 (.241)	0.999-1.002
Percent change of reticulocytes between baseline and 6th month (continuous variable)	1.002 (.507)	0.997-1.007
Percent change of Bil between baseline and 6th month (continuous variable)	1.001 (.754)	0.996-1.006
Percent change of LDH between baseline and 6th month (continuous variable)	1.008 (.032)	1.001-1.016

Bil indicates bilirubin; CI, confidence interval; HbF, hemoglobin F; HU, hydroxyurea; LDH, lactate dehydrogenase; PLT, platelets; WBC, white blood cells.

Discussion

HU is the only available drug to date that alters the process and prognosis of SCD. However, whether HU can prevent the severe chronic complications or modify the rate of mortality of SCD patients remains still an unresolved question. The present LaSHS study was designed in an attempt to answer this question by evaluating the effects of HU in a large number of SCD patients who received HU during a long treatment period, up to 17 years, and were followed in a single center. Furthermore, we compared the survival of HU patients with the survival of all other SCD patients who were followed in the same center, had similar clinical and laboratory follow-up, and received supportive care.

This prospective trial showed a dramatic reduction (> 95%) in the median annual rate of painful crisis. These results are comparable with those of the Multicenter Study of Hydroxyurea (MSH) study, in which the administration of HU was given for a much shorter median period (< 24 months).[16] The significant reduction of the painful crises, the incidence of transfusion requirements, the hospital admissions, and the incidence of chest syndrome that were observed in our study supports the notion that HU is of tremendous benefit for SCD patients and raise the issue of expanding its use in SCD syndromes.

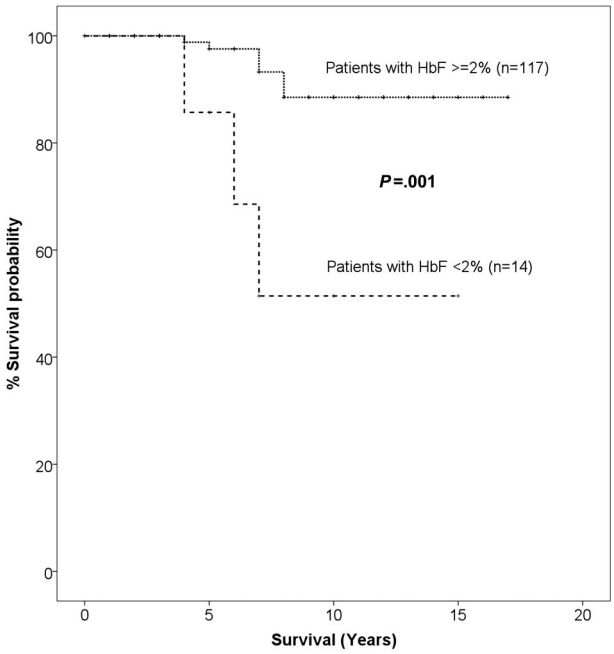

Figure 3. Patients who had hemoglobin F values of more than 2% had a 10-year probability of survival of 89%, whereas all other patients had a 10-year probability of survival of 53% (*P* = .001).

OS

The survival findings of our study were exciting, and for the first time in the literature we showed that the death rate in patients who received HU for such a long period of time was significantly lower than that observed among patients who were conventionally treated. The probability of 10-year survival was 86% for HU patients, whereas it was only 65% for patients who were conventionally treated (Figure 1). Our study also confirmed that the beneficial effect of HU on survival was mainly in HbS/HbS and HbS/β^0-thal patients (Figure 2), whereas this effect on HbS/β^+-thal was present but statistically insignificant (Figure 2C). Interestingly, in HbS/HbS the probability of 10-year survival was only 10% in patients who were treated with supportive measures compared with 100% of HU patients. Even if the number of HbS/HbS patients was not high in our study, this result strongly supports the beneficial effect of HU on survival of these patients.

Our study was not randomized. However, the survival benefit of HU-treated patients is further magnified if one considers that before starting HU, patients had significantly greater frequency of painful crises, greater number of admissions to hospital per year, greater transfusion requirements, and increased intensity of hemolysis than patients who were not given HU. Indeed, patients who did not receive HU had underlying molecular mutations of the β-gene that permit the production of HbA and thus they had milder clinical

Table 6. Multivariate analysis (Cox regression model) in HU patients

Variable	Odds ratio (*P*)	95% CI
HbF (> 2%)	0.239 (.040)	0.061-0.937
Percent change of LDH between baseline and 6th month (continuous variable)	1.009 (.036)	1.001-1.017

CI indicates confidence interval; HbF, hemoglobin F; HU, hydroxyurea; and LDH, lactate dehydrogenase.

Table 7. Factors studied in the univariate analysis for predicting survival in patients who did not receive HU

Variable	Odds ratio (*P*)	95% CI
Age, y	0.984 (.427)	0.930-1.039
Hemoglobin	0.744 (.001)	0.620-0.892
Hemoglobin F	0.923 (.027)	0.861-0.991
White blood cell counts	1.000 (.017)	1.000-1.000
Platelet counts	1.000 (.502)	1.000-1.000
Reticulocyte counts	1.056 (.003)	1.019-1.095
Bilirubin	1.313 (< .001)	1.160-1.486
LDH	1.001 (.052)	1.000-1.002

All parameters were evaluated as continuous variables.

CI indicates confidence interval; HU, hydroxyurea; and LDH, lactate dehydrogenase.

features (32.6% with HbS/IVSI-110 and 28.4% with HbS/IVSI-6). In the largest study published to-date (the MSH study), survival was similar between patients who received HU and placebo, although HU patients with moderate or severe SCD had also better survival than patients who did not receive HU.[16] In that study, which included only HbS/HbS patients, the overall reduction in mortality up to 9 years of observation was 40%. In our study, the overall reduction in mortality up to 17 years of follow-up was 73%. These results must be interpreted cautiously as our study was not randomized but we have to stress again that SCD patients who did not receive HU were also followed-up every 4 to 8 weeks in our center under the clinical care of the same treating physicians.

Predictors for survival

It is well known that HU is associated with increased levels of HbF in SCD and that increased levels of HbF improve survival.[3,10] In our study, all HU patients responded to therapy and their median HbF levels were almost 5-fold greater at 6 months compared with baseline values. Our multivariate model also confirmed that HbF is an independent predictor for survival in both SCD patients who receive and who do not receive HU. Similarly, in the MSH study, levels of HbF 0.5 g/dL or greater associated with a nearly 50% reduction in mortality,[16] whereas in that study half of the patients had no significant increments in HbF despite significant amelioration of the disease in the treatment group.

This impressive increase in HbF levels observed in LaSHS compared with the respective increase of HbF in the MSH trial may be the result of the large number of HbS/β-thal patients of LaSHS. The feeling of better amelioration of general clinical condition of patients under HU in the MSH study is in accordance with our study in which HU patients have reported a better performance status from the first month of HU administration, although this has not been confirmed by a quality of life questionnaire-based measurement. This clinical benefit to HU may also be associated with the reduction of other prognostic factors of SCD, such as leukocyte and reticulocyte counts.[10,17] In the MSH study, low leukocyte and reticulocyte counts were directly associated with low crisis rate among HU patients but not in placebo patients.[16] However, in our study we found a prognostic value of leukocytes only in patients who did not receive HU (and only in the univariate analysis) and not in HU patients; this difference may be attributable to the different study population between the 2 studies.

Table 8. Multivariate analysis (Cox regression) in non-HU patients

Variable	Odds ratio (*P*)	95% CI
HbF	0.883 (.021)	0.794-0.981
Bilirubin	1.278 (.023)	1.034-1.580

All parameters were evaluated as continuous variables.

CI indicates confidence interval; HbF, hemoglobin F; HU, hydroxyurea.

Our LaSHS study revealed that another predictor for survival with independent prognostic value was the percentage change of serum LDH between baseline and 6-month values. It has been published that patients with more severe chronic hemolysis have the greatest prevalence of pulmonary hypertension, priapism, leg ulcers, and cerebrovascular disease.[18] LDH, which serves as a surrogate biomarker of intravascular hemolysis, may also identify the clinical subphenotypes of hemolysis-associated vasculopathy.[19] Serum bilirubin had also an independent prognostic significance in patients who did not receive HU in our study. In a report by Kato et al,[19] bilirubin was also associated with vasculopathy, severe chronic complications, and increased death probability in patients with SCD.

The effect of HU on chronic complications of SCD

The increased morbidity of SCD is mainly attributable to the progressive organ damage after sickling-induced infarction. Stroke is one of the most devastating complications of SCD. In LaSHS, we had a 2.3% death-rate in HU patients as the result of intracerebral hemorrhage, which is the main cause of stroke in adult SCD. At the same time, the death rate as the result of a stroke was 2-fold greater among patients treated with supportive care (5%). Our results are similar with those of Ohene-Frempong et al,[20] who reported a prevalence of cerebrovascular accident of 4% in SCD patients. In the MSH study, cerebrovascular mortality occurred more frequently among patients originally assigned to HU than placebo,[16] but this was not related to the use of HU.

Chronic liver abnormalities are also frequent in SCD and have multifactorial pathogenesis, including cholelithiasis, viral damage, iron overload, and the disease itself. In our report 27% of the studied patients (84 of 309) had sickle cell hepatopathy with the type of injury to be mixed hepatocellular–cholestatic. The increase of survival in SCD during the recent years has revealed the liver insufficiency as one of the main causes of death in SCD.[21,22] Our LaSHS study demonstrated the clinical benefits of HU therapy in adult patients with "drepanocyte" liver. HU produced lower death rate as the result of liver dysfunction (0.7% vs 5%), although the incidence of liver impairment at baseline was similar between HU and non-HU patients (28.6% and 27.5%, respectively). HU also reduced dramatically the serum levels of bilirubin, LDH, transaminases and γGT and thus produced a beneficial effect on liver function. Our results are in accordance with the results of other studies that reported a high frequency of liver complications in adult SCD patients and their improvement under HU therapy.[21,23,24]

Avascular necrosis of bone (ANB) is another frequent and severe complication of SCD.[25,26] In the LaSHS study, at baseline, 12% of patients had a history of symptomatic ANB; a result comparable with that of Milner et al,[25] who found a prevalence of 10% of ANB in 2590 patients with SCD. During HU therapy, only 1 patient presented with ANB compared with 6 patients (3%) who did not receive HU and developed ANB during follow-up period.

Finally, leg ulcers have been recognized as an important and underestimated complication of SCD, with a prevalence of approximately 14%.[27-31] In LaSHS, we found no differences in terms of the incidence of leg ulcers between patients who did or did not receive HU. However, it appears that the use of HU induces ulcers mainly in patients with previous history of SCD ulcers, suggesting that HU could act in conjunction with other vascular abnormalities.[32]

Side effects of HU

Side effects of HU therapy were minimal, predictable, and easily manageable. The commonest side effects included neutropenia and thrombocytopenia. In our experience, the hematologic recovery after HU discontinuation is very rapid, and in the majority of cases it happens within 1 to 2 weeks after discontinuation of HU.

Regarding fertility, 6 male and 5 female patients, despite their long-term HU administration, managed to carry out a normal delivery. The oldest child is 12 years old as of this report, and he has a normal development with no health problems, as do all other children who were born during HU administration. These results suggest that HU is rather safe for patients who want to have a healthy delivery and are in accordance with the statement of the National Toxicology Program and the National Institute of Environmental Health Sciences in 2007[33] that "the use of HU in pregnancy does not seem to be commonly associated with adverse perinatal outcomes and that no data are available on long-term outcomes in children who were exposed in utero."

In our study we had no case of leukemogenesis or cancerogenesis, although such cases have been reported in the literature.[16,34-36] This finding is very important if one considers that the 131 patients had a total of 1161 patient-years of HU exposure. These results suggest that HU treatment in adult patients with SCD seems not increase the risk for leukemia or cancer.

In conclusion, our LaSHS study suggests that the administration of HU in adult patients with SCD for a long period of time significantly reduces the incidence of acute and chronic complications of SCD and that these patients have a survival advantage. This finding seems to be more prevalent in patients with HbS/HbS or HbS/β^0-thal patients, but patients with HbS/β^+-thal may be also benefit from HU administration. These results highlight the beneficial effect of HU, which appears to modify the natural history of SCD and raise the issue of expanding the use of HU in all patients with these conditions.

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Authorship

Contribution: E.V. designed and performed research, analyzed data, and wrote the paper; D.C. performed statistical analysis and wrote the paper; A. Bilalis, E.P., K.V., and G.S. followed the patients and analyzed the data; K.S. and A. Balassopoulou performed research and analyzed data; D.L. designed research and followed the patients; and E.T. performed research, analyzed data, and wrote the paper.

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