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Impact of Hydroxyurea therapy on albuminuria among children with sickle cell anemia

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An abstract of
A thesis submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Master of Science in Clinical Research

2016

ABSTRACT

Impact of Hydroxyurea therapy on albuminuria among children with sickle cell anemia

By Sarah Tehseen

Background: The process of renal damage in sickle cell anemia (SCA) is progressive and leads to end stage renal disease in 12% of adult sickle cell patients. Hydroxyurea (HU) is known to successfully reduce many complications of sickle cell disease. However, its role in sickle cell nephropathy remains uncertain. **Objectives**: To investigate the effect of HU on progression of sickle cell nephropathy as estimated by urine albumin to creatinine ratios (ACR) in children with HbSS/Sβ0 phenotype. AIM 1: Compare the urine ACR levels post- vs. pre-initiation of HU among patients with SCA. AIM 2: Among SCA patients with albuminuria, compare the urine ACR levels between SCA patients on HU and age-matched SCA patients not on HU. **Methods:** This retrospective cohort study was conducted in SCA patients tertiary care children's hospital between 2010 and 2013, with up to 2 years of follow-up. The main outcome was urine ACR. Other covariates included age, mean hemoglobin levels, indices of HU compliance and blood pressure. A paired *t* test or appropriate non-parametric test was used to compare urine ACR levels post- vs. pre-initiation of HU. An independent sample *t* test was used to compare change in urine ACR levels between exposed and unexposed patients. Multiple linear regression models were used to investigate the impact of HU therapy on change in ACR while adjusting for other covariates (AIM 2). **Results:** AIM 1: The final number of patients included was 81. Urine ACR levels were significantly lower ~2 years after initiation of HU compared to levels pre-HU (7±15 two years post HU vs. 11.4±24 pre-HU; p=0.02 N=65). This change was most pronounced among patients who had abnormal ACR levels before initiation of HU (N=21; 23.4±39 2 years post-HU vs. 95±103 pre-HU; p=0.02). AIM 2: The final number of patients included was 48. HU therapy was associated with a significant reduction in ACR over ~1 year of follow up compared to patients not on HU, controlling for age and baseline hemoglobin. **Conclusion:** HU therapy in SCA is associated with lower urine ACR levels over time.

Keywords: Sickle cell anemia, hydroxyurea, albumin to creatinine ratio.

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ACKNOWLEDGEMENTS

The author would like to thank the following for their insightful input in the preparation of this thesis: M Yee, C Joiner, P Lane, V Barry, C Kempton, W Woods, TR Ziegler, M Klein and A Mantunga.

The author would also like to acknowledge the invaluable support of her family (Husband Anees, Son Zakariya, parents and parents-in-law), thesis committee, MSCR program colleagues and Pediatric Hematology Oncology fellowship program, all of whom have made this endeavor possible.

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INTRODUCTION

Sickle cell disease (SCD) is an inherited hemoglobinopathy that affects 90,000-100,000 Americans, mainly of African American or Hispanic heritage. This makes SCD one of the most common genetic diseases in the U.S. minority population [1]. The management of SCD has undergone major advances over the last 2-3 decades, which has led to improved survival of individuals into adulthood. Given advances in survival, these individuals face consequences of chronic end organ damage, such as chronic kidney disease (CKD) [2].

Renal damage is a common but underdiagnosed complication of SCD. The process of renal damage in SCD begins in childhood and is progressive through adolescence and adulthood, leading to end stage renal disease (ESRD) in 4-18% of individuals with severe sickle genotypes HbSS/Sβ0 thalassemia [sickle cell anemia (SCA)] in adult life. Since ESRD is an independent predictor of mortality in SCD, it is crucial to prevent renal injury in initial stages before irreversible damage occurs [3].

Standard methods for monitoring of renal function such as estimation of glomerular filtration rate (GFR) via serum creatinine-based formulas are not reliable in SCD and are of little utility in screening and diagnosis of sickle cell nephropathy, as serum creatinine remains low in SCD until very late stages of renal damage, when irreversible renal damage has already occurred. Currently the commonly used screening tool for early renal damage in sickle cell patients is albuminuria, as determined by urine albumin to creatinine ratios (ACR) [4]. According to international consensus guidelines defining chronic kidney disease (KDIGO), chronic albuminuria meets the criteria for diagnosis of CKD, regardless of kidney function [5]. Multiple studies have demonstrated a higher prevalence of

albuminuria in sickle cell patients compared to normal population, and the severity and prevalence of albuminuria increases with age in patients with SCA [6, 7].

Hydroxyurea (HU) is the only medication approved by the FDA for long-term management of SCD. Its mechanism of action involves increase in fetal hemoglobin with a reduction in sickled hemoglobin, leading to a reduction in frequency of vaso-occlusive events. Previous studies have shown improved quality of life with fewer hospital admissions associated with chronic use of HU [8]. The most recent National Heart, Lung and Blood (NHLBI) foundation guidelines recommend the use of HU therapy in all individuals with HbSS SCD, regardless of disease severity [9]. However, data regarding the impact of HU therapy on renal damage in sickle cell disease are inconclusive, emphasizing the need for further studies to estimate its effect. [13, 15, 16, 19] This retrospective cohort study aims to investigate the effect of HU therapy on urine ACR levels among children with SCA.

BACKGROUND

Renal damage in SCD

Current literature regarding development and progression of sickle cell nephropathy suggests that renal damage begins in early childhood, nearly universally in children with SCA. The hypertonic acidic media of renal medulla promotes RBC sickling within the vasa recta of nephron. This sickling leads to ischemia and infraction of renal medulla which manifests itself as loss of urine-concentrating ability during early childhood [10, 11]. The ischemia also leads to a release of vasodilators such as prostaglandins and nitric oxide, which in turn lead to glomerular hyperfiltration. Over time, glomerular hyperfiltration leads to glomerular hypertrophy and, eventually, glomerulosclerosis with nephron loss [3]. The ongoing glomerular damage leads to microalbuminuria, microalbuminuria, and a gradual decline in GFR over time [10]. The majority of SCD patients have some degree of albuminuria (61-79%) in adult life and 4-18% of these patients will go on to develop ESRD [12]. The mean survival of SCD patients after ESRD diagnosis is 4 years and 40% die within 20 months of starting dialysis, highlighting the need to detect and treat nephropathy in early stages [2, 13].

Benefits and limitations of the use of albumin to creatinine ratio (ACR)

Measurement of urine ACR levels is a cheap and practical method for population screening of nephropathy. In other diseases associated with nephropathy like diabetes, albuminuria is predictive for progression to ESRD [14]. However, its correlation with CKD in SCA has not been well established. Also, urine ACR levels are known to undergo transient changes based on time of the day and other variables like fever, pain etc. Despite these limitations,

ACR on spot urine specimens is considered the standard for detection of early nephropathy in SCD. Patients are diagnosed with microalbuminuria if they have abnormal urine ACR (>=30 mg/g-300 mg/g) in two of three spot urine specimens taken consecutively over three months and also verified with first morning urine specimen.[5].

Evidence for use of HU therapy

Multiple studies have investigated the effect of hydroxyurea on sickle cell nephropathy (SCN), but their interpretation is complicated by the use of differences in the parameters used for estimation of renal damage and different study populations. A brief review of important studies and their limitations are provided below.

The Baby HUG study was an NIH-sponsored placebo-controlled randomized controlled trial that studied the effect of HU on renal parameters like serum creatinine, glomerular filtration rate (GFR), diethylenetriaminepentaacetic acid clearance (TcDTPA) and renal ultrasound. This study was conducted in infants with a mean age of 18 months, with a follow-up period of 24 months. It showed a decrease in renal hypertrophy (estimated via ultrasound) and improved urine-concentrating ability in individuals on HU. There was no significant difference in DTPA derived GFR values. However, the study population was very young and follow-up period was short and limited to early childhood, which does not provide information about long-term impact of HU therapy on renal function. This is an important consideration since sickle cell nephropathy often leads to morbidity and mortality in adolescents and adults rather than children. Also, estimated GFR equations may not be reliable for the determination of renal function in SCD [15].

The Hydroxyurea Study of Long Term Effects (HUSTLE) was a prospective study looking at multiple renal parameters: urine microalbumin, measured GFR, and cystatin C levels. The study population included 23 children with a mean age of 7.5 years who were prospectively followed for 3 years. Among these patients, HU therapy was associated with a significant decline in glomerular hyperfiltration; however, no difference was found in urine microalbumin levels. Though this study was prospective and had data on urine ACR, the sample size was small and study participants were not compared to SCA patients not on HU therapy [16].

Multiple small and medium-sized cross-sectional studies have shown a lower prevalence of albuminuria among patients on HU therapy, both in pediatric and adult age groups; however, these studies only used one ACR measurement per patient and did not capture the trend of albuminuria over time [17] [18] . Furthermore, many of these studies investigated the effect of multiple therapies that can potentially modify albuminuria (ACE inhibitors, transfusion) on renal function at the same time and did not look at the independent effect of HU [13, 19].

A recent study prospectively examined the impact of HU therapy on urine ACR among 58 adults with SCA. Among those adults, use of HU for six months was associated with a significant decline in urine ACR levels, which were most obvious among patients with albuminuria. However, this study only looked at HU therapy effect at 6 months and did not take pediatric population into account. Current models of sickle cell nephropathy suggest that renal damage in SCA worsens with increasing age. Hence it is crucial to study the impact of interventions like HU in pediatric age group, where the potential for modulation of sickle cell nephropathy is much higher [20].

Other potential correlates of ACR

Urine ACR levels among children with SCA are affected by multiple other factors. One of the factors that have consistently shown a positive correlation in multiple studies with urine ACR levels is age [6, 12]. Certain red cell and hemoglobin indices have also shown association with urine ACR levels in literature. These include hemoglobin and fetal hemoglobin, which have shown strong negative correlations with urine ACR levels in many previous studies [7, 17]. Increased hemolytic activity (as measured by reticulocyte count or lactate dehydrogenase levels) has shown a strong positive association with ACR levels [17]. Higher blood pressure is also considered to be associated positively with ACR levels; however, data defining this relationship are scant in pediatric population [21, 22].

Rationale

The gaps in study of the effect of HU therapy on SCN include the lack of use of well-defined renal parameters (like urine ACR) in children to study kidney damage and small sample sizes, as well as lack of medium to long term follow up in pediatric population.

To address these gaps, this study investigated the impact of HU therapy on sickle cell nephropathy (as determined by urine ACR levels) in a retrospective fashion among patients with severe genotypes of sickle cell disease including SS and Sβ0 thalassemia (SCA). We compared the urine ACR post- vs. pre-initiation of hydroxyurea, so that patients were their own comparison group.

In addition to determining the trend of urine ACR levels among all SCA patients on HU therapy, we focused on the effect of HU therapy among patients with albuminuria. This was a significant consideration as this subgroup of patients is particularly vulnerable to

development of chronic kidney disease in adult life and hence would benefit most from therapies potentially modifying early renal disease. In order to examine this effect, we compared changes in urine ACR post versus pre HU among SCA patients with albuminuria only prior to initiation of HU therapy. Among SCA patients with albuminuria while on long term HU, we determined change in ACR and compared it to SCA patients with albuminuria not on HU. Comparison to unexposed patients enabled us to account for the ACR trend in absence of HU and helped establish the role of HU on early sickle nephropathy as determined by urine ACR levels.

METHODS

AIMS and Hypothesis

The overall aim of this study was to examine the effect of HU therapy on albuminuria among patients with sickle cell anemia (SCA) using urine ACR levels. The effect of *introduction* of HU was explored as well as chronic ongoing changes in ACR in patients who have been on HU for 6 or more months

Primary AIM 1: In Among children with SCA, determine estimate the change in albuminuria urine ACR (ΔACR) that occurs over time with the introduction of HU therapy. This change will also be estimated independently in the subgroup of patients with albuminuria pre HU.

Sub AIM: Examine the association of urine ACR levels with age at start of HU therapy, baseline hemoglobin (Hb.), fetal hemoglobin (HbF), marker of hemolysis (reticulocyte count) and blood pressure (reported as mean arterial pressure MAP).

Primary AIM 2: Among patients with SCA, who have albuminuria *while being on HU*; determine longitudinal change in ACR over 1-2 years. Compare ΔACR between patients on HU versus SCA patients with albuminuria who are not on HU or other sickle cell therapies.

Hypothesis AIM 1: In children with SCA, levels of urine ACR will decrease following the introduction of HU therapy.

Hypothesis AIM 2: In children with SCA with albuminuria while on stable long term HU therapy, the urine ACR levels will decrease over time compared to patients not on HU (unexposed) and with albuminuria.

Study design

Retrospective cohort study of SCA patients who started HU between 2011-2013 followed at Children's Healthcare of Atlanta. (Figure 1a and 1b)

Time zero AIM 1: Day of start of HU therapy

Time zero AIM 2: Day of first recorded abnormal ACR

Study Population:

Children's Healthcare of Atlanta (CHOA) takes care of ~1700 pediatric patients with SCD each year. CHOA recommendations for screening for SCN went into effect in 2010 and include urine ACR testing on spot urine specimens every 12 months starting at 5 years of age in all SCD patients. However, implementation of this guideline was not universal among CHOA SCD clinics, leading to a reduction in potential sample size for this study.

The Aflac sickle cell program prospectively maintains a clinical SCD database that includes all individuals who were active SCD patients at one of the three CHOA locations. This database includes individuals' SCD genotype, past and current use of disease-modifying therapies such as HU including start and stop dates and hospital utilization data.

To identify eligible patients, this database was queried for all individuals with HbSS or Sβ0 who started HU therapy between 1/1/2011 to 12/31/2013. Electronic chart abstraction was used to verify information obtained from database and for collection of study variables.

Study population AIM 2

Exposed patients were defined as SCA patients with albuminuria while being on HU therapy for 6 or more months. Unexposed patients were defined as SCA patients with albuminuria who were not on any sickle modifying therapy. The unexposed patients were frequency matched by age (±1 year) and baseline hemoglobin (±1 gm/dl) to exposed patients and were obtained from the prospective SCD database maintained by the Sickle Cell Disease Program of CHOA.

Important definitions AIM 1

Albuminuria= Defined as Urine ACR ≥30mg/g on at least one spot urine specimen in 2 years pre or 6 months post start of HU. Microalbuminuria=Defined as urine ACR levels between 30-300 mg/g. Macroalbuminuria = Defined as urine ACR levels > 300 mg/g. Baseline hemoglobin=Hemoglobin on the day of start of hydroxyurea therapy. Start date for HU therapy: Defined as the first day medication order appeared in patient medications list with no HU in the previous year.

Important definitions AIM 2

Albuminuria: Urine ACR ≥30mg/g on at least one spot urine specimen 6 months post start of HU for exposed patients (AIM 2) and in similar calendar years (±2 years) for unexposed patients. Unexposed patients: SCA patients not on HU or other sickle modifying therapies matched by age and baseline hemoglobin to exposed patients

Eligibility criteria

AIM 1

Subjects were included if they started HU at the age of 5 years or more, had sickle cell genotypes SS or Sβ0, and had >1 year of medical data available prior to and after the HU start date (n=81). Patients were excluded (n=261) if they did not have at least one ACR value available both before and after initiation of HU therapy (N=153) or were on sickle modifying therapies like ACR inhibitors/ARB, chronic transfusion in the last 12 months or had undergone transplant (N=48) or had other sickle genotypes (N=10). . A detailed description of inclusion and exclusion process is provided in Figure 1a.

AIM 2

SCA patients on HU therapy were included if they had ACR data (2 or more values) after being on HU for 6 or more months and had albuminuria. Unexposed SCA patients were included if they had 2 or more ACR values in similar calendar years and had albuminuria.

Subjects were excluded from study if they were on disease-modifying therapies like chronic transfusions, bone marrow transplant, or treatment with angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) for both Aim 1 and 2. A detailed description of patient selection is provided in 1b.

Primary exposure

Primary exposure was defined as HU therapy taken for 6 or more months at maximum tolerated dose (maximum tolerated dose is based upon hematologic and clinical response but usually does not go beyond 30 mg/kg) based on review of individual patient charts.

Patients once started on HU therapy are continued on it unless sickle related complications arise (CVA) necessitating other therapies like chronic transfusion. Patients included in this study were verified by electronic chart review to be on chronic HU therapy.

Study variables and study procedures

Main outcome

The main outcome variable was ACR estimated on spot urine specimen for each patient. It is a continuous variable measured in mg/g. Values ≥ 30 mg/g are considered abnormal. For study AIM 1, urine ACR levels were recorded within 2 years prior to HU therapy initiation (pre HU ACR) and all available ACR values were recorded after being on HU therapy for 6 or more months. If more than 1 value of urine ACR was available prior to initiation of HU therapy, the value closest to the date of HU start was recorded.

For AIM2, all available ACR values were recorded for exposed patients after being on HU therapy for 6 or more months and in similar calendar years (± 2 years) for unexposed patients. (Figures 2a. and 2b.)

Other covariates

Other covariates of interest included age of patient in years upon start of HU therapy, as of 7/31/2014 (time of review of patient database for eligibility), systolic and diastolic blood pressure (recorded at each time point an ACR value was recorded for the patient) and CBC indices associated with use of HU therapy including hemoglobin (hb.), mean corpuscular volume (MCV), fetal hemoglobin (HbF) and reticulocyte count. Per clinical protocol, CBCs are performed every 1-3 months while on Hu therapy. We gathered retrospective

data on CBC indices on the day of start of HU therapy (baseline) and at 6 months, 12 months and 24 months post-HU therapy to assess compliance/response to HU.

These covariates were collected for exposed and unexposed patients in similar fashion as AIM 1. A diagrammatic description of data collection schema is provided (Figure 2a. and 2b.)

This study was approved by the IRB of Children's Healthcare of Atlanta.

Power calculation and statistical plan AIM 1

Power calculation

There were no available data on mean change in ACR levels over time among pediatric sickle cell patients on HU therapy in literature. Based on information obtained from 30 random sickle cell patients followed in our clinic, we were able to determine the mean difference in ACR levels pre and post HU (15 mg/g) and well as its variance (40 mg/g). Based on this information with a power of 0.80, effect size of 15 mg/g, and alpha of 0.05, the estimated number of patients we needed to recruit for our study was found to be 58. The actual number of patients found to be eligible was 81 and all were included in the study. This sample size was sufficient to detect a minimum effect size of 12 mg/g between the pre and post HU ACR values.

Statistical methods

We reported means and standard deviations for normally distributed continuous variables (age at start of HU, mean arterial pressure, hemoglobin levels, mean corpuscular volume, absolute reticulocyte count and fetal hemoglobin). We reported median and interquartile

ranges (IQR) for non-normally distributed variables. Non-normal variables were natural log transformed (*ln*) for figures, comparison, and modeling.

Wilcoxon signed rank test or paired *t* test was used to compare the difference in urine ACR levels post- vs. pre-initiation of HU therapy. A paired *t* test was also used to compare baseline CBC indices (hemoglobin, MCV, reticulocyte count and fetal hemoglobin) to CBC indices after 12 months of HU therapy to determine compliance/response to HU therapy for this cohort of patients. Chi-square test or Fisher's exact test was used to determine change in proportion of patients with albuminuria over time.

Multivariable analysis

Since all of the patients included in the study were exposed to HU, in order to determine the effect of HU therapy while adjusting for other covariates, we created indicator variables for HU (exposure) so that HU = 0 for ACR prior to HU therapy initiation and HU=1 for ACR post HU therapy (also described in figure 2a.). Assumptions of normality for the dependent outcome [*ln* (ACR)] were assessed by looking at the histogram and probability plots. We assumed a linear change in *ln (*ACR) based on individual patients' plots. Given multiple ACR values for each individual at different time intervals we used a mixed effects linear model to estimate the effect of HU therapy while adjusting for baseline hemoglobin, MAP, reticulocyte count, and age of start of HU therapy on ACR. This enabled us to estimate the change in ACR within each individual over time as well as between individuals. We also estimated the interaction effect of time from HU therapy (pre and post) with HU. The validity of model was assessed by looking at the distribution of residual plots for the dependent outcome.

Model*: ln* (ACR) = β0 (fixed intercept) + b0 (random intercept for Ln-ACR for each patient) + β1 (age at start of HU) + β2 (baseline hemoglobin) + β3 (ΔMAP) + β4 (reticulocyte count) + β5 time in years pre and post HU + β6 (HU therapy 0=time pre HU and 1=time post HU) + β7 (Time*HU) + E

Power calculation and statistical plan AIM 2

Power calculation

There were no data on comparison of change in mean/median ACR (ΔACR) among SCA patients with albuminuria on HU therapy versus not on HU therapy in literature. Among the 20 patients randomly selected from sickle cell clinic, 5 were found to have albuminuria (two patients on HU and 3 not on HU). Based on those patients, the effect size for ΔACR difference between exposed and unexposed patients was determined to be 61 and SD difference of 70. With a sample size of 48 (actual number of subjects obtained from review of data) our study had a power of 84% to detect a difference of 61 mg/g between the exposed and unexposed patients.

Statistical plan

We reported means and standard deviations for normally distributed continuous variables (Age in years, MAP, Hb, MCV, absolute reticulocyte count and HbF). We reported median and IQR for non-normally distributed variables (ACR). Non-normal variables (ACR) were log-transformed for comparison and modeling.

Paired *t* test or Wilcoxon signed rank test was used to compare change in ACR levels over time in the exposed and unexposed groups. Wilcoxon rank-sum test was used to compare difference in ΔACR between the exposed and unexposed patients. Chi-square test or

Fisher's exact test was used to determine change in proportion of patients with albuminuria over time in exposed and unexposed patients.

Multivariable analysis

A linear regression model was used to determine the effect of HU therapy on change in urine ln(ACR) levels while adjusting for other covariates like age, baseline hemoglobin, reticulocyte count (marker of hemolysis) and mean arterial pressure. Since the two factors consistently associated with ACR are age and hemoglobin, we matched our unexposed group based on these variables and also adjusted for them in the multivariable analysis.

$$\Delta\ ln\ (\text{ACR}) = \beta 0 + \beta 1\ \text{HU} + \beta 2\ (\text{age}) + \beta 3\ (\text{baseline Hemoglobin}) + \beta 4\ (\text{baseline reticulocyte count}) + \beta 5\ (\text{Mean Arterial Pressure}) + E$$

A p value of 0.05 was considered to be significant for all comparisons. The data analysis was conducted in SAS v. 9.4.

Missing data on outcome of interest

The most common missing variable was ACR especially before start of HU. Lack of missing ACR data led to exclusion of patients from final study sample. The guidelines for testing urine ACR on sickle cell patients in Children's Healthcare of Atlanta were not rigorously followed in 2010-2012 and hence many patients did not have data on ACRs. This trend has slowly improved since 2014. For AIM 1, since all patients started HU therapy, the missing data did not vary by exposure. For AIM 2, there were a greater number of unexposed patients with missing ACR data than exposed. A detailed description of patient selection process is provided in figures 1and 1b.

Missing data on other covariates

Patients who had missing data on other covariates of interest were included in the study and the missing variables were imputed to their mean for inclusion in multivariable analyses after verifying that results with and without imputation were not significantly different.

RESULTS

AIM 1

There were 81 patients with SCA ranging in age from 5-22 years who were included in the study. There were 65 (80%) with 2 years of ACR (two or more values) data after starting HU and 20% (N=16/81) patients had only 1 year of ACR (one ACR) data post HU therapy. 48% of study population (N=39/81) had 2-3 years of ACR data. Since univariate analysis of patients with 1 year of data only (20%) and those with two or more years of ACR data (80%) were similar, table 2, figure 3 and figure 4 only shows analysis of patients with 2 years of outcome data. However, all the patients were included in the multivariable analysis using mixed effect model.

The descriptive characteristics of the whole study population are shown in table 1. The mean age of start of HU therapy was 10.4±3.9 years and baseline hemoglobin was 8.3 g/dl. Median ACR values pre-HU were 11.4±24 mg/g. 26% of population (21/81) had abnormal ACR prior to start of hydroxyurea therapy. The only non-normal variable in this cohort of patients was ACR, which was then natural log-transformed for comparison, modeling and graphics.

Table 2 shows results of paired comparison of median ACR values before and after initiation of HU therapy for patients with 2 years of ACR data (N=65/81). As shown in table 2, median ACR values showed a decline after a mean time of 1±0.5 years of HU therapy but the difference was not significant (11.6±25 mg/g vs. 9±21 mg/g p-value=0.08). However median ACR values were significantly lower after HU therapy for a mean time

of 1.8±0.6 years compared to pre HU values (11.6±25 mg/g vs. 8±17 mg/g p-value=0.007). Figure 3 shows changes in mean *ln* (ACR) values during hydroxyurea therapy over 2 years.

The percentage of patients with albuminuria declined from 27% (N=18/65) prior to initiation of HU therapy to 21% (14/65) after 1 year of HU therapy and the difference was not statistically significant (P-value 0.40). However, the percentage of patients with albuminuria declined to 14% (N=9/65) after 2 years of HU therapy and it was significantly lower than the percentage with pre-HU albuminuria (P-value: 0.04). Figure 4 shows the change in proportion of patients with albuminuria over time.

Among those with normal ACR levels pre HU (N=47/65), 8% of the cohort had new onset of albuminuria after 1 year of HU therapy (N=4/47) and 4 % (N=2/47) had persistence of albuminuria after 2 years of HU therapy. There was a significant increase in hemoglobin (8.3±1.2 vs. 9.4±1.5 g/dl p-value 0.02) MCV (85±12 vs. 93±12 p-value <0.001) and fetal hemoglobin (9.4±5 vs. 16.3±8.4 p-value 0.001) after 12 months of HU therapy. There was a significant decrease in reticulocyte count (331±120 vs. 274±121) after 12 months of HU therapy.

Multivariable analysis AIM 1

Figure 5 shows the overall trend of *ln* (ACR) levels for this study population over follow up period. The results of linear mixed effects model showed a significant negative association of HU therapy with *ln* (ACR) levels while adjusting for age at start of HU therapy and baseline hemoglobin. While the overall trend of *ln* (ACR) was negative over time, we did not find a significant interaction between time and HU therapy. Reticulocyte count and mean arterial pressure were not found to have a significant association with *ln*

(ACR) and did not affect association of *ln* (ACR) with HU and other covariates and were hence excluded from final model. High baseline hemoglobin was associated with significantly lower urine ACR levels. The results of mixed effects model are provided in table 3. The residuals of *ln* (ACR) for the mixed effects model showed a random normal distribution.

Subgroup analysis (Patients with albuminuria pre HU)

There were 21 patients who had albuminuria prior to starting HU (26%). 90% of these patients (N=18/21) had 2-3 years of follow up data after starting HU therapy and 3 patients had only 1 year of follow up ACR data (N=3/21). Since the results of univariate analysis of patients with 1 vs. 2 or more years of outcome data were similar, table 5 and figures 6 and 7 show comparison for patients with at least 2 years of follow up data only (N=18). However multivariable analysis employed all the patients (N=21)

Table 4 shows comparison of baseline descriptive statistics between the patients with normal ACR versus albuminuria pre HU. Patients with albuminuria had significantly lower hemoglobin levels (7.6±1.2 vs.8.5±1 p-value: 0.04) and higher MCV values at baseline (90±8.3 vs.83±13.3 p-value=0.02). The remaining indices including age, HBF, MAP and reticulocyte count were similar between the two groups.

Table 5 shows the results of paired comparison of median ACR values pre- and post-HU therapy. Median ACR values showed a significant decline at 1 and 2 years of HU therapy compared to pre HU values (70±115 vs. 32±48 at 0.8±0.5 years p-value=0.005 and 24.5±39 at 2±0.5 years p-value =0.02, . Figure 6 shows change in mean ln(ACR) values over 2 years of HU therapy.

The proportion of patients with abnormal ACR declined to 55% (N=10/18) over a mean follow-up time of 0.8±0.5 years (P-value: 0.001) and to 39% (N=7/18) over a mean follow-up of 2±0.5 years (p-value 0.007) on HU therapy. Figure 7 and table 5 show the change in proportion of patients with abnormal ACR over time.

Multivariable analysis subgroup

Figure 8 shows the overall trend of *ln* (ACR) values for this study population. The results of linear mixed effects model (table 6) showed a significant negative association of HU therapy with *ln* (ACR) levels while adjusting for age at start of HU therapy and baseline hemoglobin. While the overall trend of ln (ACR) was negative over time, we did not find a significant interaction between time pre and post HU therapy start and HU therapy itself. Reticulocyte count and mean arterial pressure were not found to have a significant association with *ln* (ACR) and did not affect the associations of HU with *ln* (ACR) in a meaningful manner. Hence they were excluded from final model. Older age at start of HU therapy was associated with significantly higher urine ACR levels while accounting for time. The residuals of *ln* (ACR) for the mixed effects model showed a random normal distribution.

Characteristics of cohort of patients excluded due to missing ACR data

The number of patients who started HU between 2011 and 2013 and were reviewed for eligibility was 341. 58% (N=153) of the cohort of patients reviewed for eligibility had no ACR data before starting HU therapy and were thus excluded. In order to determine if the subset of patients excluded due to missing outcome data was different from patients included in the study we compared mean age of start of HU, baseline hemoglobin and

median ACR values post HU (if available) for excluded cohort with patients included in the study. The patients excluded due to missing ACR data (N=153) had similar age at start of HU therapy (10.2±3.57 vs. 10.4±3.9) and had similar baseline hemoglobin (8.2±1.09 vs.8.2±1.07). 6% (N=8/143) of the excluded group had no ACR data. The median ACR for the rest of the excluded cohort was 10±7.5 mg/g, which was lower than patients included in the study. 11 % (N=16/143) of patients who were excluded due to incomplete ACR data but had 1 or more ACR values post HU (no pre HU values) had abnormal ACR compared to 25% included in the study. Eight of the patients with albuminuria post HU who were excluded for AIM 1 met eligibility criteria for AIM 2 were included in AIM 2 of study.

AIM 2

The total number of SCA patients with first recorded abnormal ACR (after the age of 5 for unexposed and after being on HU ≥ 6 months for exposed) albuminuria identified for inclusion in the study was 48. Among those patients, 58% (N=28/48) were exposed (on HU) 42% (N=20/48) were unexposed. All of these patients had two ACR values over 1-2 years of follow up.

Table 7 shows the comparison of baseline characteristics of exposed and unexposed patients with albuminuria. There was no significant difference in mean age, baseline hemoglobin or other CBC indices between the two groups. Baseline fetal hemoglobin was high among unexposed patients but the difference was not statistically significant. First recorded median ACR values were similar between the two groups. Age of start of HU therapy for exposed patients was 10±4.5 years (vs. calendar age of 8.2±5 years). ACR

values for exposed patients were obtained at mean time of 1.1±0.9 years and 1.9±0.8 years from start of HU therapy.

Table 8 shows results of comparisons of median ACR values in the exposed and unexposed patients. Among the unexposed patients, the median ACR values did not show a significant decline at mean time of 1±0.8 years (54±88 vs. 57±212 mg/g, p-value 0.3) There was a significant decline in median ACR values among exposed patients over 0.8±0.5 years of follow up (62.5±51 vs. 25.2±42 mg/g, p-value 0.003). There was a significant difference in ΔACR values between the exposed and unexposed patients as shown in table 8 (-25±56 vs. -6.4±24 p-value 0.04). The change in Ln-ACR values over time for exposed and unexposed patients is shown in figure 9.

The proportion of exposed patients with albuminuria changed from 100% to 46% (N=13/28) over ~1 year of follow up and the decrease was significant (p-value <0.0001). The proportion of unexposed patients with albuminuria changed from 100% to 75% (N=15/20) at ~1 year of follow up and decrease was significant (p-value 0.02). The changes in proportion of patients with albuminuria among the exposed and unexposed patients are provided in figure 10.

Multivariable analysis AIM 2

The result of multiple linear regression models (table 11) showed a negative effect of HU therapy on change in *ln* (ACR) levels while adjusting for age and baseline hemoglobin. Reticulocyte count, blood pressure and fetal hemoglobin did not have a significant association with *ln* (ACR) and did not affect association of *ln* (ACR) with other covariates. Hence they were excluded from final multivariable model.

DISCUSSION

Progressive renal damage is a significant cause of morbidity and mortality among adult SCA patients, making it crucial to prevent or delay this progression during childhood. Hydroxyurea therapy has proven to be an effective tool in management of sickle cell disease leading to significant reduction in hospital utilization and overall improvement in survival and QOL of SCA patients [8]. However, evidence regarding its impact on sickle nephropathy has been inconsistent. [13, 15, 16, 19]

In our study we found that HU therapy was associated with lower urine ACR levels. This association was evident with post- vs. pre-HU ACR comparison (AIM 1) and when ΔACR was compared between the exposed and unexposed patients (AIM 2). Among patients with normal ACR pre HU, the number of patients who developed albuminuria was very small. Among patients with abnormal ACR pre HU, albuminuria resolved in a significant number after 6 or more months of HU therapy. The results of mixed effects multivariable analysis revealed a statistically significant association of lower urine ACR levels over time with HU while adjusting for age and baseline hemoglobin. When patients with albuminuria on HU were compared to unexposed patients, the multivariable analysis revealed a statistically significant association of HU with lower ACR while adjusting for age and baseline hemoglobin.

The proportion of pediatric and adolescent patients with albuminuria in our study was similar to previous literature [7]. The proportion of patients with albuminuria was lower with the use of HU. This association was observed both when compared to proportions pre HU and when compared to unexposed patients. The findings of our study were similar to

previous small cross-sectional studies performed in children and adults with SCA that showed a lower prevalence of albuminuria among SCA patients on HU [13, 18]. However, those studies did not take multiple ACR values or change in ACR into account. Also, these studies investigated the effect of multiple therapies (ACE inhibitors, chronic transfusion) on albuminuria at the same time and not the independent effect of Hydroxyurea which can potentially lead to a bias in true estimation of the effect of HU [19].

A recent prospective cohort study of adult SCA patients showed a significant decline in quantitative ACR levels among adults on HU therapy for six months or more. This finding is similar to our data which also indicated that that long term HU therapy was associated with lower urine ACR[20].

However, our findings are in contrast to the well-known multicenter Hydroxyurea Study of Long Term Effects (HUSTLE project), in which HU therapy was not shown to have a significant impact on albuminuria but did show decreases in glomerular hyperfiltration [16]. One of the possible reasons could be the older mean age of our cohort at the time of start of HU (10 years versus 7 years) where renal damage had progressed to manifest albuminuria compared to patients in HUSTLE study where the patients only had glomerular hyperfiltration. That age discrepancy can also explain the very small number of patients with microalbuminuria (N=4) at the start of HU therapy in HUSTLE project which may be underpowered to detect difference in albuminuria given small sample size.

High baseline hemoglobin showed a strong negative correlation with ACR levels for the whole cohort of patients for AIM 1. However, high baseline hemoglobin did not show a significant association with ACR among individuals with albuminuria prior to HU therapy

(sub group Aim 1). Since the patients with albuminuria pre HU had a significantly lower baseline hemoglobin compared to patients with normal albumin excretion, it is likely that the association of hemoglobin with ACR for the whole cohort was driven by the subgroup of patients who had albuminuria and became non-significant among the subgroup. Association of hemoglobin with urine ACR has been well described in previous literature. [21, 22]

Among patients with albuminuria prior to initiation of HU therapy (subgroup AIM 1), older age at the start of HU was associated with higher ACR levels. Increase in ACR with increasing age has been well documented in patients with sickle cell disease and correlates with worsening renal damage [6, 7]. However, no study to our knowledge has looked at the association of age of start of HU therapy with ACR levels. The finding from our study suggest a possible protective benefit of early initiation of HU therapy towards albuminuria however future studies are needed to ascertain this possible advantage.

None of the patients included in our study were diagnosed with hypertension. We were not able to find a significant association of urine ACR levels with blood pressure which is in contrast to a previous observational study[21] in pediatric population.

The mechanism by which Hydroxyurea therapy modulates sickle nephropathy has not been well defined. Multiple studies in pediatric and adult population have shown an association between high ACR levels and markers of hemolysis, thereby implying that endothelial damage related to chronic hemolysis in SCD plays a crucial role in development of SCN [23] . Ongoing hemolysis in SCD leads to release of Heme and Heme Oxygenase 1. Heme Oxygenase 1 causes vasodilatation and subsequent hyperfiltration whereas Heme itself is

pro inflammatory and promotes vascular injury. [24] . Since use of Hydroxyurea therapy leads to a significant decrease in hemolysis by increasing fetal hemoglobin and reducing the amount of sickled hemoglobin, it could potentially lead to a decrease in progressive vascular damage, which can halt the ongoing kidney damage. We were not able to demonstrate a significant correlation between ACR and reticulocyte count in our cohort of patients; however, we did see a significant decrease in hemolysis (as evident by reduced absolute reticulocyte count) after 6-12 months of HU therapy. Another potential though theoretical mechanism of action can be direct effect of HU on podocyte structure and function through proliferation markers like Cyclin D 1 (CD1) [25]. HU can lead to destabilization of those proliferation markers and prevent aberrant podocyte proliferation which can prevent glomerular damage and development of FSGS (Focal Segmental Glomerulosclerosis) [24] [26]. Further studies are needed to determine this effect.

Our study examines the independent effect of HU therapy on albuminuria in a longitudinal fashion by comparing pre and post HU data where patients were their own comparison. Among patients with albuminuria on stable long term HU therapy, we also compared ACR over time between exposed and unexposed patients and showed a significant decline in ACR over ~1year among patients on HU. Our study contributes to the literature by its longitudinal design and comparison of multiple time points of ACR data. The findings from our study suggest a very promising role of HU therapy towards prevention and modulation of albuminuria in conjunction with previous literature.

Our study had multiple limitations given its retrospective design and the number of subjects excluded from final sample due to missing ACR data. The result of analysis of excluded patients with missing or insufficient outcome (ACR) data revealed a similar age and

baseline hemoglobin to included cohort however among excluded patients who had one ACR value only or no pre HU ACR data, median ACR levels and proportion of patients with albuminuria were lower. Also there is a potential confounding by indication since patients on HU therapy were different (more severe phenotype) from SCA patients not on HU therapy. Other limitations include unmeasured confounding variables which could not be accounted for given study design and misclassification of ACR as its measurements can undergo transient changes.

Indications for HU therapy have evolved over the last few years. Hydroxyurea HU therapy was only initiated in SCA patients with severe disease phenotype (multiple VOC, hospital admissions, acute chest syndromes) prior to 2013. However, it is now considered for all SCA patients 12 months and older regardless of disease severity, based on recommendations from the multicenter Baby HUG study. This led to an increase in the number of patients on HU and change in characteristics of population on HU to include younger age and less severe phenotype which may lead to a difference in association of HU with ACR in new population. Since the ACR data in our patient population had a skewed distribution, it could be possible that the sample size estimated were not as reliable as they would be for a normally distributed outcome.

There is a lack of high quality evidence regarding optimal treatment for sickle cell nephropathy. Currently the two most commonly suggested therapies are HU, ARBs and ACE inhibitors. While ACE inhibitors and ARBs have shown a significant reduction in albuminuria among adult SCD patients, their role in long-term management of SCD remains unclear. Use of ACE inhibitors in SCA patients has also been limited due to development of hyperkalemia in some cases [13]. Other sickle-modifying therapies, like

early initiation of chronic transfusion therapy, have also shown association with a lower prevalence of albuminuria in a small subset of SCA patients. [27]. However, HU therapy offers a better quality of life and would be more feasible for prevention of early sickle nephropathy. Hence further studies with prospective design and longer follow-up periods are needed to conclusively determine the role of HU in progression of sickle cell nephropathy. Also crucial in our understanding of nephropathy is the development of newer early biomarkers of nephron damage which will improve our understanding of early renal damage in sickle cell disease and will aid in determining the potential effect of sickle modifying therapies in very early stages [28].

Conclusion

Hydroxyurea therapy is associated with significantly lower quantitative ACR levels as well as the proportion of patients with albuminuria over 6 months to three years of follow up. Future prospective studies with longer duration of follow-up will aid in better understanding of the role of HU in sickled kidneys. Discovery of novel biomarkers of nephron damage will also aid in better comprehension of early nephropathy in SCA, which will eventually lead to development of therapies designed to curb sickle cell nephropathy in very early stages.

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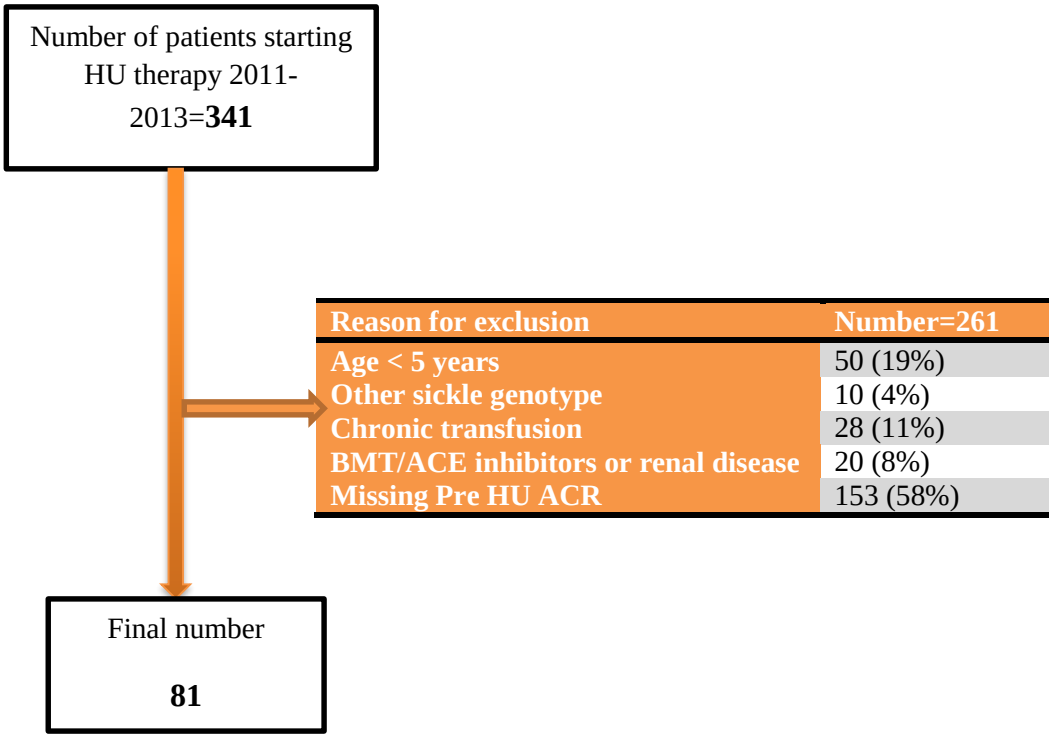
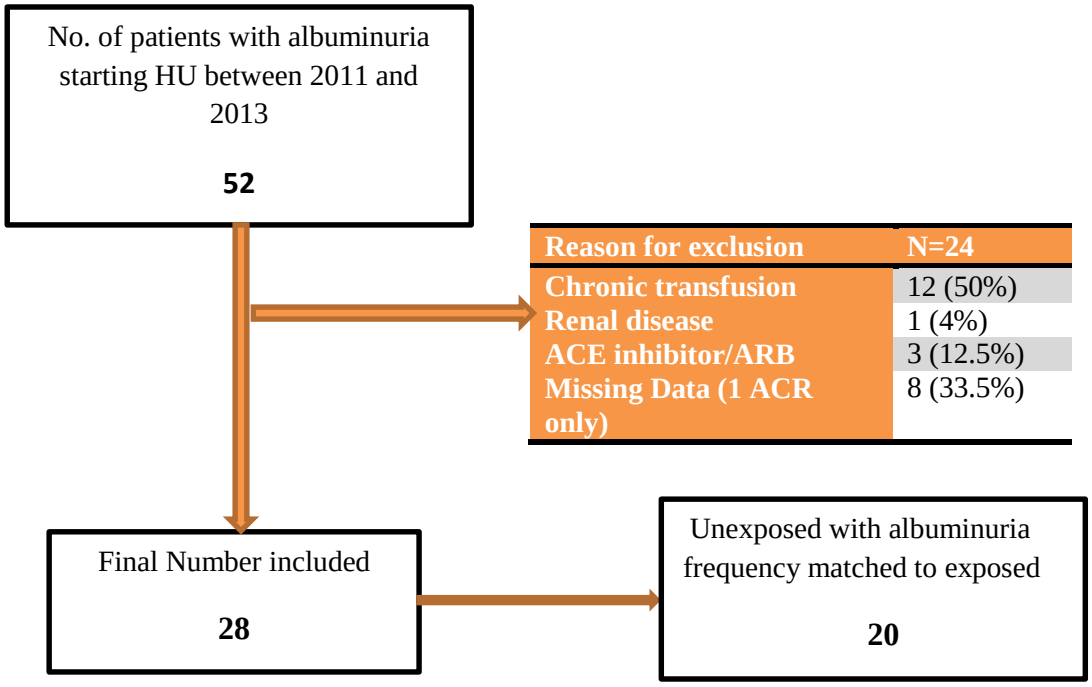
FIGURES AND TABLES

Figure 1A: Flow sheet of patient selection for Aim 1



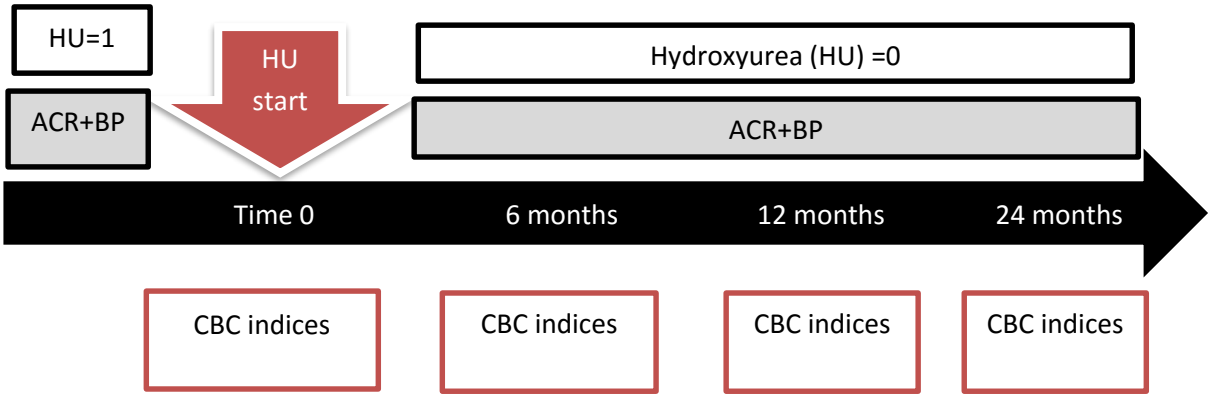
Reason for exclusion	Number=261
Age < 5 years	50 (19%)
Other sickle genotype	10 (4%)
Chronic transfusion	28 (11%)
BMT/ACE inhibitors or renal disease	20 (8%)
Missing Pre HU ACR	153 (58%)

Figure 1B: Flow sheet of patient selection for Aim 2



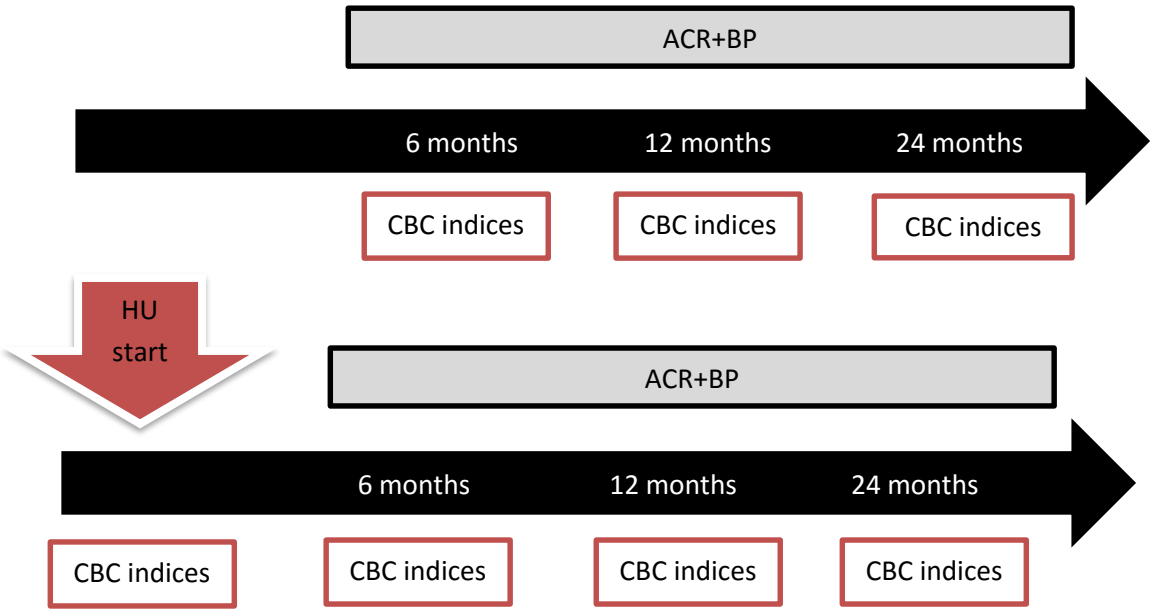
Unexposed patients with SCA were matched by age (±1 year) and Hemoglobin (±1 gm) to exposed SCA patients. SCA=Sickle cell Anemia (SS/Sβ0 Thalassemia)

Figure 2A. Data collection schema Aim 1



For each patient HU=0 indicated ACR values pre HU and HU=1 ACR values post HU therapy initiation

Figure 2B: Data collection schema Aim 2



HU=Hydroxyurea, ACR=Albumin to creatinine ratio, BP=Blood Pressure, CBC indices=Hemoglobin (Hb.) Mean corpuscular volume (MCV), Fetal hemoglobin (HbF) and Reticulocyte count.

Time Zero AIM 1=Start of HU therapy

Time zero AIM 2 = Day of first recorded ACR (only patients with abnormal ACR)

RESULTS AIM 1

Table 1: Baseline characteristics of SCA patients who started Hydroxyurea (HU) between 2011 and 2013 (N=81)

Variables	Values
Age (years) Mean±SD	10.4± 3.9
MAP (mmHg) Mean±SD	79.3 ± 8.5
Hemoglobin (g/dl) Mean±SD	8.34 ± 1.2
MCV (fl/cell) Mean±SD	86.4 ± 12.8
Reticulocyte count (cells/mm^3) Mean±SD	331 ± 120
Hemoglobin F Mean±SD	9.4 ± 4.6
ACR (mg/g) Median±IQR	11.4 ± 24
Gender N (%)	M=37 (46%) F=43 (54%)
Abnormal ACR pre HU N (%)	21 (25%)

Age= Age at the time of start of Hydroxyurea; MCV=Mean corpuscular volume; ACR=albumin to creatinine ratio MAP: Mean arterial pressure Hemoglobin F=Fetal hemoglobin;

Table 2: Comparison of median ACR values pre and post HU for patients with 2 years of data (N=65)

	Pre HU	Post HU 1	P-value (Pre vs. post1)	Post HU 2	P-value (Pre vs. post 2)
ACR mg/g (Median±IQR)	11.6±25	9.0±21.5	0.17	8±17	0.007
Abnormal ACR N (%)	18(28%)	14(21%)	0.40	9 (14%)	0.04
Time pre and post HU(years) Mean±SD	-0.5 ±0.7	0.8±0.4		1.9±0.6	

Abnormal ACR = ACR >=30 mg/g. P-values for ACR (mg/g) comparisons reflect Wilcoxon signed rank test; for Abnormal ACR % reflects chi-square test. Time=Time in years pre and post HU

Table 3: Results of multivariable analysis of the effect of covariates on *Ln(*ACR) using mixed effects (N=81)

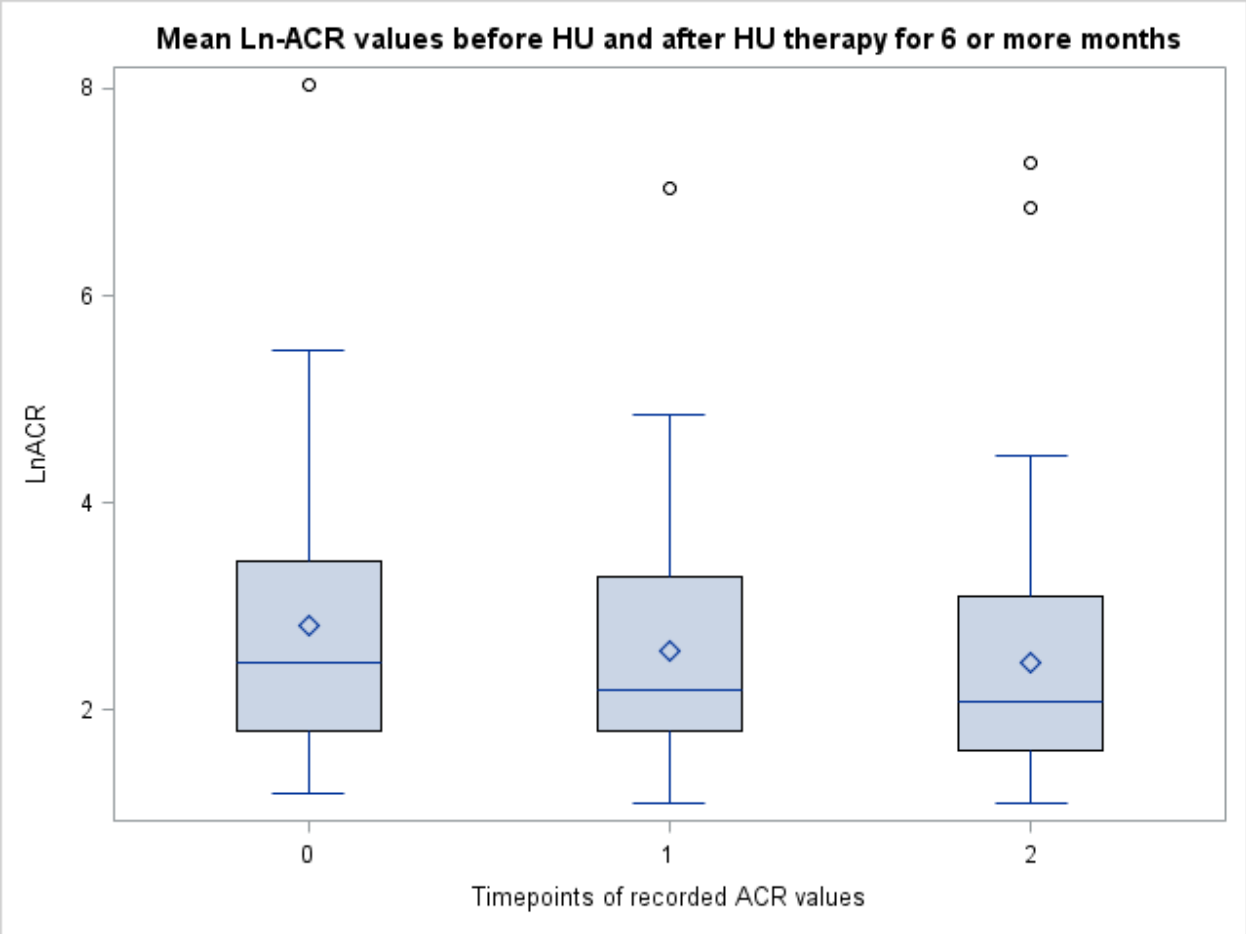
Model: *ln* (ACR) = β 0 + b0 (random intercept) + β 1(HU) + β 2 (age) + β 3 (HU) + E
Where for each patient HU=0 (ACR pre HU) and HU=1 (ACR post HU)

	Estimate of difference in *ln* (ACR)	**95% confidence interval**	**P-value**
HU therapy	-0.27	-0.45-(-0.07)	0.0008
Baseline Hemoglobin	-0.27	-0.45-(-0.10)	0.003
Age (yrs.)	0.04	-0.02-0.09	0.20

Age=Age at start of Hu therapy, baseline hemoglobin=Hb on the day of start of HU therapy

Interpretation: Being on HU therapy was associated with a decrease in ACR of 1.3 mg/g (anti-log of estimate of Ln-ACR), compared to pre-HU ACR, adjusting for age and baseline hemoglobin.

Figure 3. Change in mean *ln* (ACR) values over time (N=65)



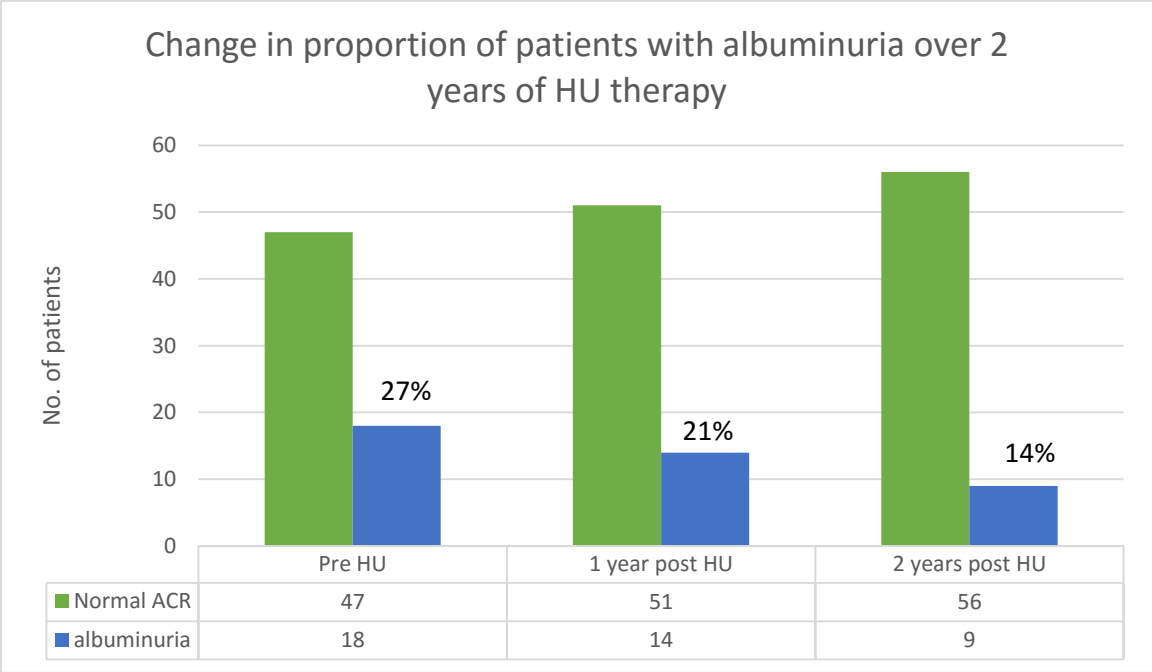
Time 0 = *ln* (ACR) values before start of HU therapy

Time 1 = *ln* (ACR) values after ~1year of HU therapy

Time 2= *ln* (ACR) values after~2 years of HU therapy

The *ln* (ACR) values showed a non-significant decrease at time 1 compared to time 0 (2.80±1.3 vs. 2.60±1.10 p-value 0.06) and a significant decrease at time 2 (2.45±1.2 p-value of 0.01) using paired *t* test 05

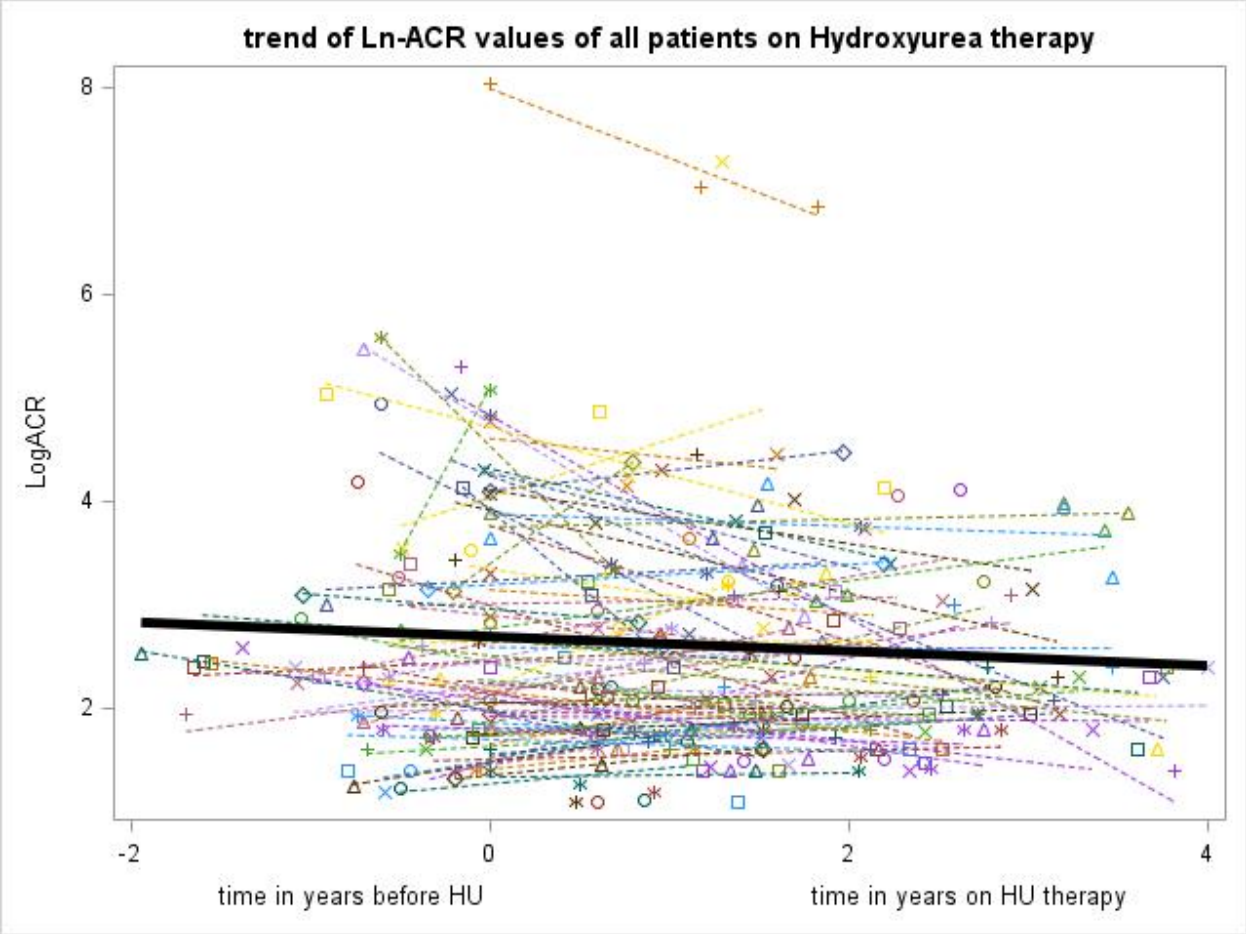
Figure 4. Proportion of patients with albuminuria over 2 years of HU therapy (N=65)



The decrease in proportion of patients with albuminuria was not significant at 1-year post HU (P-value=0.40) but was significant at 2 years post HU (P-value=0.04)

Albuminuria = Urine ACR >=30 mg/g

Figure 5. Individual patient trajectories showing change in *ln* (ACR) levels after starting HU therapy (N=81)



RESULTS SUBGROUP AIM 1 (PATIENTS WITH ALBUMINURIA PRE HU)

Table 4: Comparison of baseline characteristics of SCA patients (normal vs. albuminuria group)

Variables	Normal ACR pre HU (N=59)	Abnormal ACR pre HU (N=21)	P-value
Age yrs. (Mean±SD)	10.3±6.5	10.5±6.7	0.86
Baseline Hb. g/dl (Mean±SD)	8.5±1.0	7.6±1.2	0.004
MCV fl/cell (Mean±SD)	83±13.3	90±8.3	0.02
Reticulocyte count (cells/mm^3) (Mean±SD)	331±129	331±91	0.98
MAP (mmHg) (Mean±SD)	79±8.6	80±8.4	0.61
ACR mg/g (Median±IQR)	9.3±7.4	95±103	<0.0001
Hemoglobin F(Mean±SD)	9.7±4.6	8.3±4.7	0.27
Gender N (%)	M=37(46%) F=43(54%)	M=10(48%) F=11(52%)	

MCV: Mean corpuscular volume, MAP: Mean arterial pressure, ACR: Albumin to creatinine ratio. P-values (age, Hb, MCV, reticulocyte count, MAP and hemoglobin F) =Independent sample t test; ACR = Wilcoxon sum rank test). Baseline Hb=Hemoglobin recorded on day of start of HU therapy

Table 5: Comparison of median ACR pre and post HU-among SCA patients with albuminuria pre HU (N=18)

	Pre HU	Post HU 1	P-value (Pre vs. post1)	Post HU 2	P-value (Pre vs. post 2)
ACR mg/g (Median±IQR)	70±115	32±48	0.005	24.5±39	0.004
Abnormal ACR N (%)	18(100%)	10(55.5%)	0.001	7 (39%)	0.007
Time pre and post HU (years) Mean±SD	-0.26 ±0.3	0.8±0.5		1.85±0.7	

Abnormal ACR = ACR >=30 mg/g. P-values for ACR (mg/g) comparisons reflect Wilcoxon signed rank test; for Abnormal ACR % reflects chi-square test

Table 6. Result of multivariable analysis of the effect of HU on *ln* (ACR) using a mixed effects model among patients with albuminuria pre HU (N=21)

Model: *ln* (ACR) = β 0 + b0 (random intercept) + β 1(HU) + β 2 (age) + β 3 (baseline Hb) + E

	Estimate of *ln* (ACR)	**95% Confidence interval**	**P-value**
HU therapy	-1.05	-1.52-(-0.6)	<0.0001
Age (yrs.)	0.11	0.03-0.2	0.006
Baseline Hemoglobin	-0.11	-0.42-0.2	0.60

Age=Age at start of Hu therapy, baseline hemoglobin=Hb on the day of start of HU therapy

Interpretation: Initiation of HU therapy was associated with a decrease in ACR by 3 mg/g (anti-log of estimate of Ln-ACR) while adjusting for age and baseline Hemoglobin when compared to pre HU ACR. Patients who had older age of HU start by 1 year had significantly higher ACR by 1.13 mg/g while adjusting for other covariates

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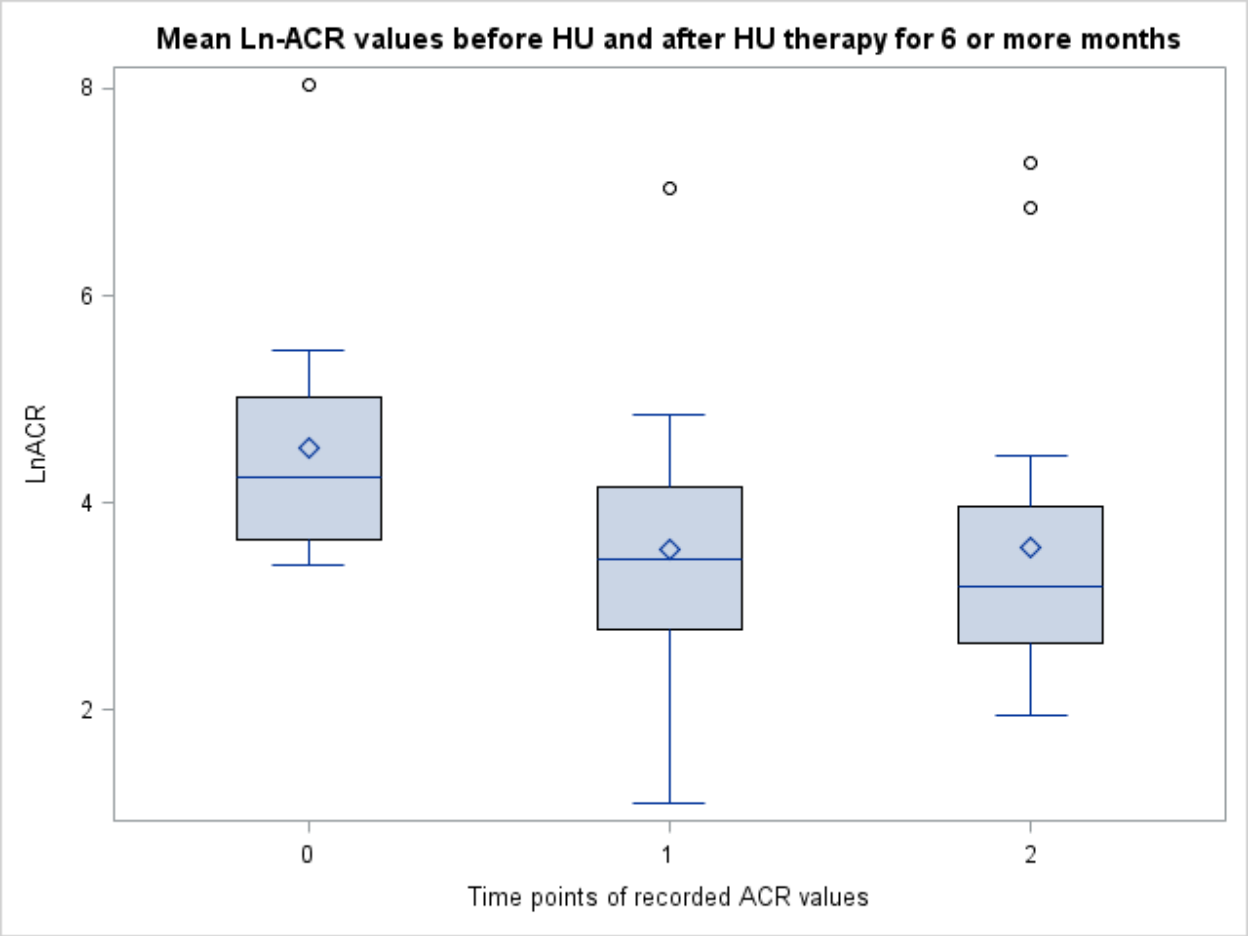
Figure 6: Changes in mean *ln* (ACR) values on Hydroxyurea (HU) therapy among patients with albuminuria pre HU (N=18)



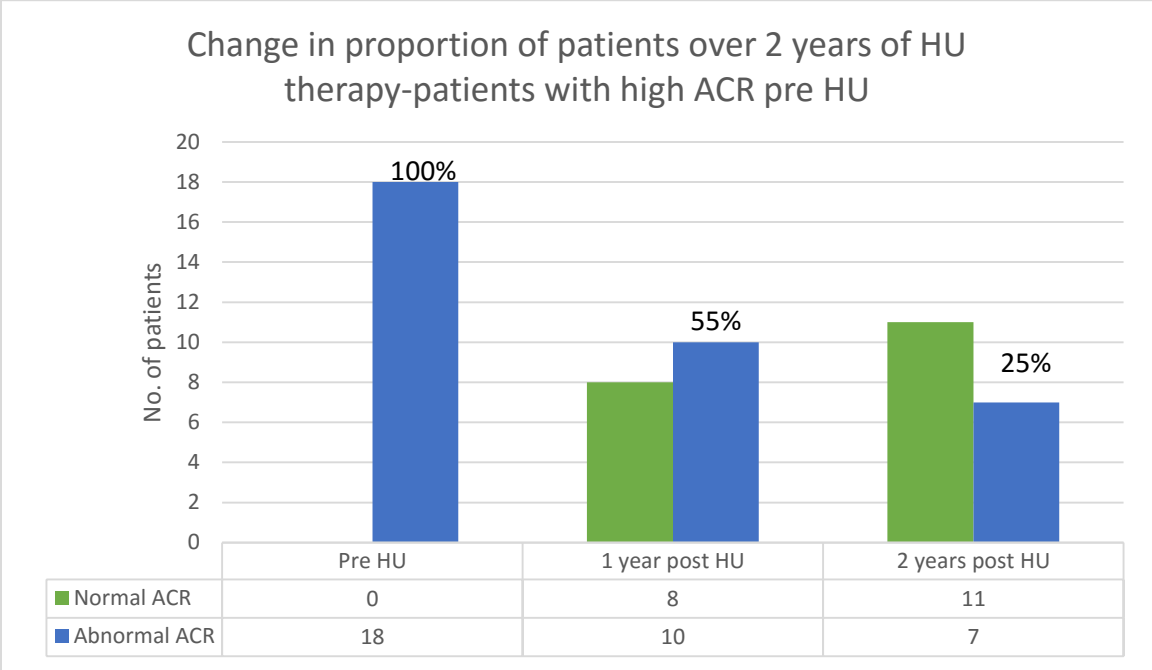
Time 0 = *ln* (ACR) values before start of HU therapy

Time 1 = *ln* (ACR) values after ~1year of HU therapy

Time 2= *ln* (ACR) values after ~2 years of HU therapy

The Ln-ACR values showed a significant decrease at time 1 (4.53±1.10 vs. 3.54±1.3 p-value 0.002) and 2 (3.5±1.4 P-value: 0.02) using paired *t* test compared to time 0

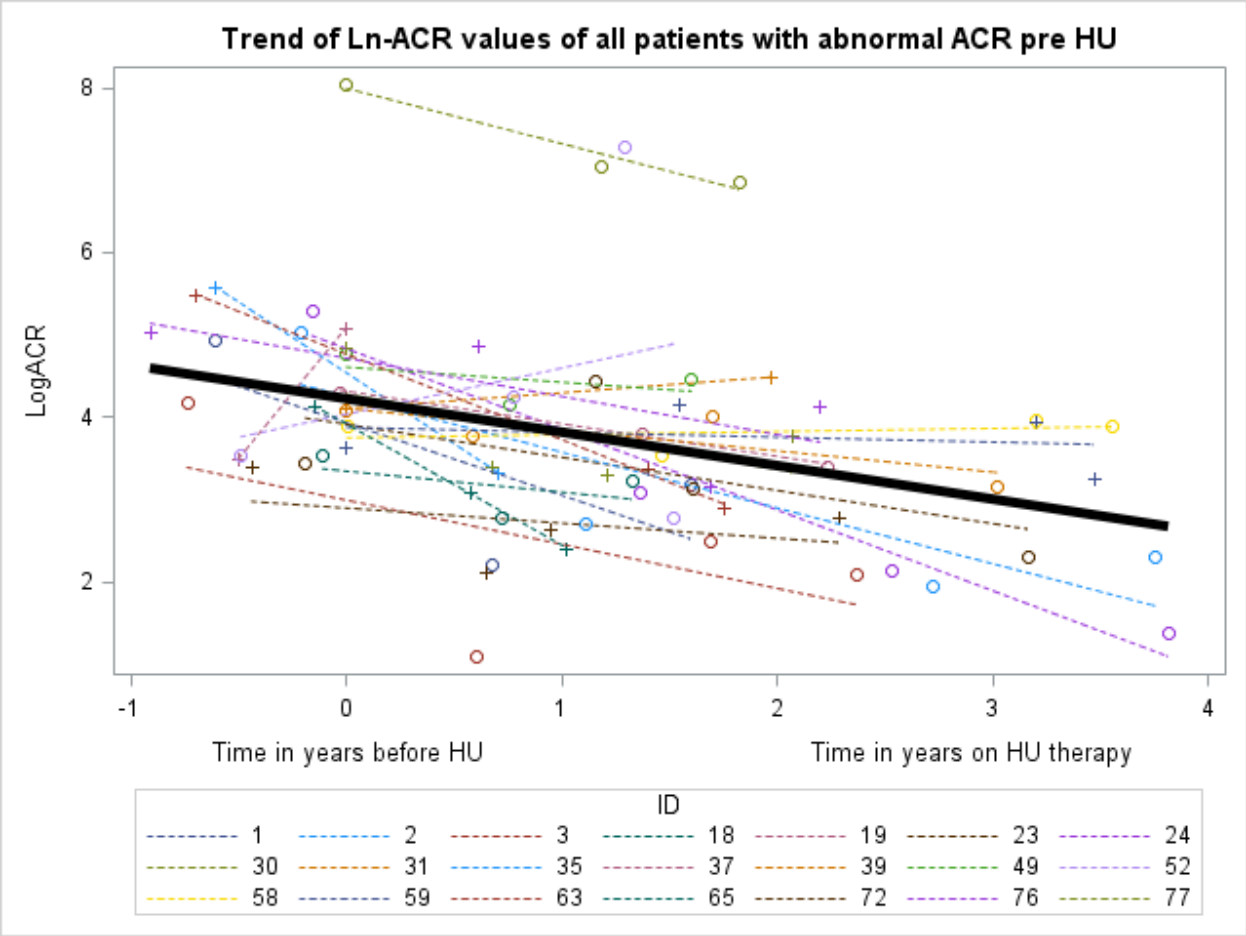
Figure 7: Proportion of patients with albuminuria over 2 years of HU therapy (N=18)



The decrease in proportion of patients with albuminuria was significant at ~1-year post HU (P-value=0.001) and at ~ 2 years post HU (P-value=0.007)

Albuminuria = Urine ACR >=30 mg/g

Figure 8. Individual patient trajectories showing change in *ln* (ACR) levels after starting HU therapy (N=21)



RESULTS AIM 2

Table 7: A comparison of baseline characteristics of SCA patients with albuminuria exposed versus unexposed to hydroxyurea therapy for at least 6 months (N=48).

	Exposed (N=28)	Unexposed (N=20)	p-value
Age (years) Mean±SD	12±4.6	11.8±4.2	0.94
Baseline Hb (g/dl) Mean±SD	8.0±1.2	8.2±1.1	0.60
MAP (mmHg) Mean±SD	78.7±8.0	77.2±9.0	0.61
Gender N (%)	F=9(45%)	F=12 (43%)	0.90
MCV (fl/cells) Mean±SD	90.5±8.1	87.3±8.2	0.20
Reticulocyte count cell/mm^3 Mean±SD	355±117	404±158	0.25
Fetal Hemoglobin Mean±SD	11±5.4	8.0±4.5	0.06
ACR (mg/g) (Median±IQR)	62.5±51	54±88	0.50

Age=Age as of 7/30/2014 (date of patient review). MAP=Mean arterial pressure. Baseline Hb=Hemoglobin at the time of start of HU therapy for exposed and in similar timeframe for unexposed, MCV=Mean corpuscular volume. ACR=Albumin to creatinine ratio. Comparisons for Age, MAP, Hb, MCV, Reticulocyte, and Fetal Hemoglobin use independent sample t test. Comparison of ACR use Wilcoxon Sum rank test and Gender use Chi-square test.

Table 8: A comparison of change in ACR values (ΔACR) over ~1 year exposed versus unexposed (N=48)

	HU therapy (N=28)	No HU (N=20)	P-value
1st ACR (Median±IQR)	62.5±51	54±88	
ΔACR (Median±IQR)	-26±56	-6.4±42.5	0.04
2nd ACR (Median±IQR)	25.2±42	57±212	
Time interval b/w ACR 1 and 2	0.80±0.5	1.10±0.8	

ΔACR =Difference in ACR over ~1 year. P-value comparison of ΔACR exposed versus unexposed employ Wilcoxon Sum rank test.

Table 9: Result of univariate analysis of the association of covariates with difference in *ln* (ACR) values among SCA patients (N=48)

	Estimate of Δ *ln* (ACR)	**Confidence Interval**	**p-value**
Age (years)	0.03	-0.03-0.09	0.40
HU (Y vs. N)	-0.82	-1.4-(-0.25)	0.005
Baseline Hb	-0.30	-0.55- (-0.06)	0.02
Reticulocyte Count	-0.0005	-0.003-0.002	0.20
MAP (mmHg)	0.02	-0.02-0.05	0.80

Hydroxyurea therapy is associated with a greater decrease in urine ACR levels by 2.5 mg/g (antilog of estimate of Diff Ln-ACR for HU). Higher baseline hemoglobin by 1 gm. /dl is associated with lower in ACR levels by 1.5 mg/g (antilog of estimate of Δ *ln* (ACR) for Hb)

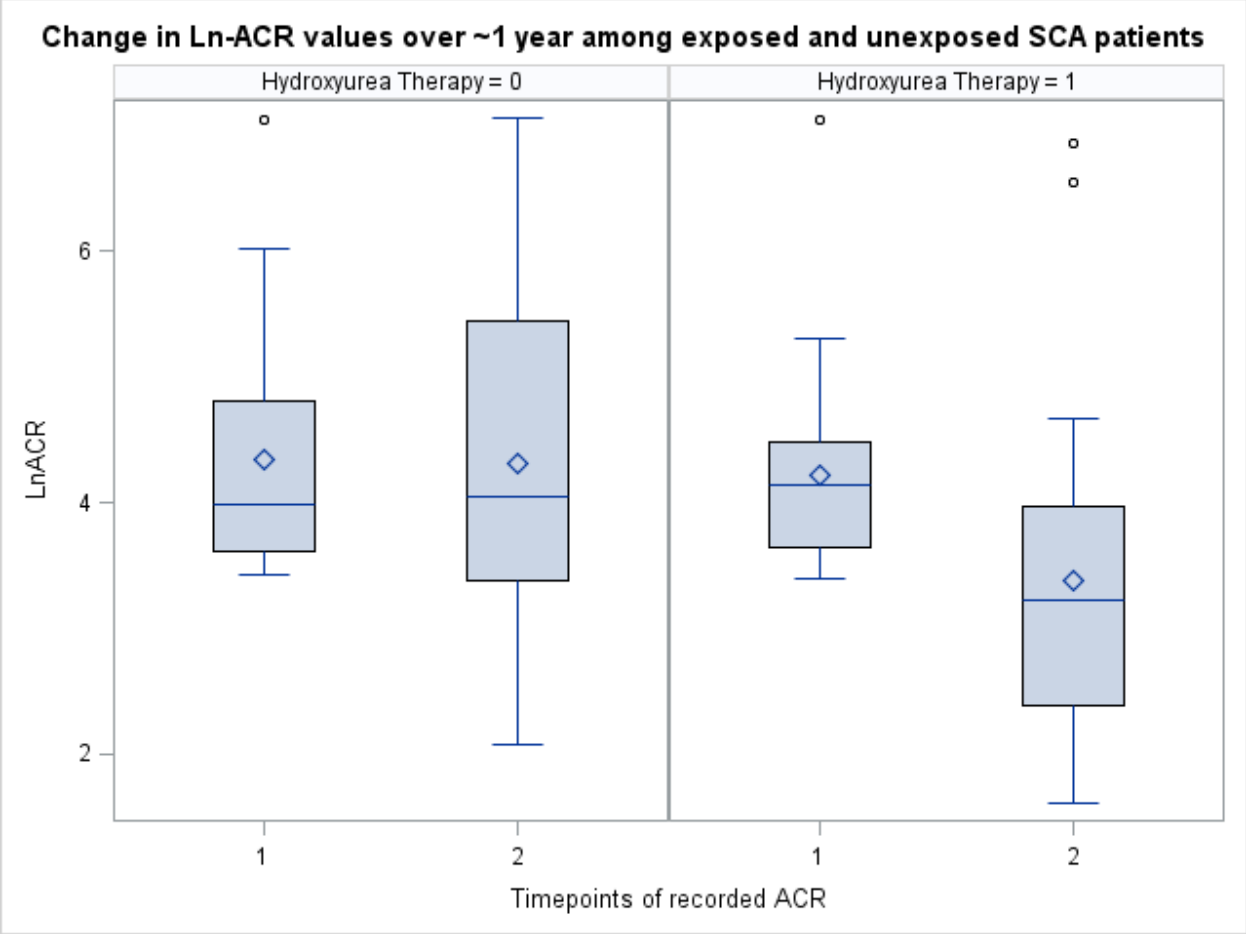
Table 10: Result of multivariable analysis of the effect of HU therapy on Δ *ln* (ACR) values among SCA patients while adjusting for covariates (N=48)

	Estimate of Δ *ln* (ACR)	**Confidence Interval**	**p-value**
HU	-0.90	-1.4-(-0.4)	0.002
Baseline Hb	-0.40	-0.61- (-0.14)	0.02
Age (years)	0.04	-0.02-0.1	0.40

Interpretation

Hydroxyurea therapy is associated with a greater decrease in urine ACR by 2.5 mg/g (antilog of estimate of Δ *ln* (ACR) while adjusting for baseline hemoglobin and age.

Figure 9: Changes in mean *ln* (ACR) values over ~1 year among patients with and without HU therapy (N=48)

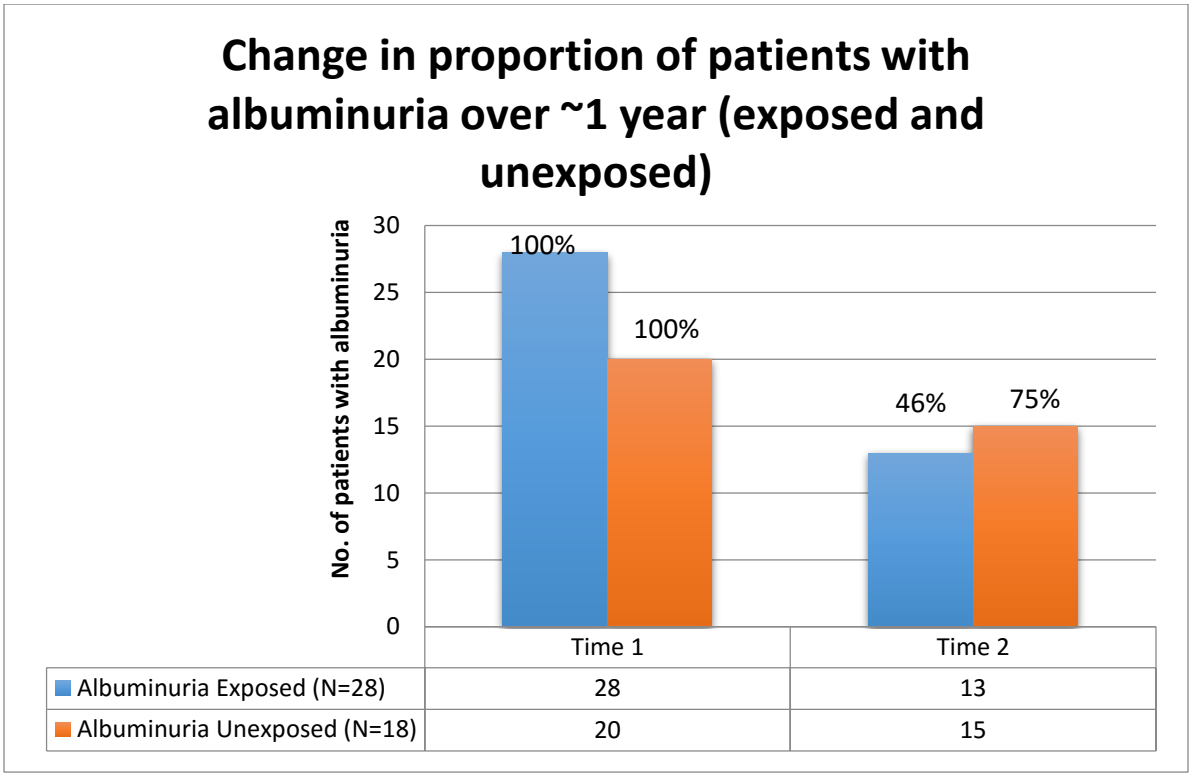


HU=1 Exposed; *ln* (ACR) levels decreased significantly after ~1 year (4.27±0.70 vs. 3.40±1.3; p-value=0.0002)

HU=0 Unexposed; *ln* (ACR) levels did not change significantly after ~1 year 4.33±1.01 vs. 4.30±1.30; p-value 0.90)

Time 1=Time of first recorded ACR (≥ 6 months of HU for exposed and in similar time for unexposed)

Figure 10: Change in proportion of patients with albuminuria over ~ 1 year among exposed (on HU) and unexposed (no HU)



There was a greater decrease in proportion of patients with albuminuria among exposed (P-value <0.0001) versus unexposed (P-value 0.02) though the decline was significant in both groups