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ORIGINAL RESEARCH



# The use of hydroxyurea in the real life of MIOT network: an observational study

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**ABSTRACT**

**Background:** Hydroxyurea (HU) has been widely used in clinical practice to manage patients with non-transfusion dependent thalassemia (NTDT). Few data are available about the effects of its administration in Italian patients. We assessed hematological and non-hematological outcomes following short- and long-term exposure to HU.

**Research design and methods:** We considered 71 NTDT patients (30 females) enrolled in the Myocardial Iron Overload in Thalassemia Network and treated for >12 months with HU.

**Results:** The mean duration of HU treatment was 8.23±5.79 years, starting at a mean age of 37.02 ±12.06 years. A significant increase in hemoglobin and mean corpuscular volume values and a down-regulation of all erythropoietic and/or hemolysis indices were detected after at least 12 months of treatment. In 28 patients the hemoglobin increase was ≥1.0 g/dl, associated with a higher HU dose. The hematological response dropped in long-term treatment. A favorable impact of HU treatment in limiting the progression of several complications typical of NTDT syndrome was observed.

**Conclusion:** Our findings seemed to suggest that in several NTDT patients HU could be still a valid option to limit the advance in overall disease clinical burden without carrying significant adverse events and increase in mortality.

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**KEYWORDS**
Adverse events; hemoglobin; hydroxyurea; thalassemia

## 1. Introduction

Hydroxyurea (HU) is an oral and well-known chemotherapeutic drug that ameliorates the severity of β-thalassemia by increasing the production of the γ-globin, which pairs together with α-globin chains to form fetal hemoglobin (Hb F) and consequently decreases the α/β-chain imbalance [1]. Due to this activity, HU has been widely employed in patients with transfusion-dependent thalassemia (TDT) and with non-transfusion dependent thalassemia (NTDT) to increase hemoglobin and to reach transfusion independency, respectively [2,3]. However, there are several caveats concerning the use of HU in the management of patients with thalassemia. First, HU response has always been unpredictable and highly variable. An in vitro evaluation of HU response with the use of primary erythroid cultures was developed to predict the patient's response to HU in vivo [4,5]. Furthermore, other relevant problems were the optimal HU dosage to be used and the desensitization to HU following the long-term treatment in vivo, as observed also in vitro [4,5].

Current NTDT clinical guidelines indicate HU as a treatment option, not only to increase Hb F and total Hb, but also to manage specific clinical complications in patients with NTDT [6,7]. In clinical practice of several thalassemia centers, HU has been spontaneously administered for the management of patients with NTDT since long time, independently from clinical trials and even before the publication of NTDT guidelines. Nevertheless, there are few studies addressing both a long-term administration and the use of HU in the management of Italian patients with NTDT [4,5,8,9].

Against this background, we assessed the clinical efficacy and safety of HU in NTDT patients with short- and long-term exposure enrolled in the large Myocardial iron Overload in Thalassemia (MIOT) network, which collects laboratory, radiological and clinical data and reflects the therapeutic approaches performed in Italy.

## 2. Patients and methods

### 2.1. Study population

The MIOT project was an Italian network built in 2006 and constituted by 68 thalassemia centers and 9 Magnetic Resonance Imaging (MRI) centers that performed MRI exams for cardiac and hepatic iron overload assessment using homogeneous and standardized procedures [10,11]. All centers were

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linked by a web-based database, where clinical and instrumental data were recorded from birth and updated at every MRI, performed by protocol every 18 months.

Seventy-nine non-transfusion dependent β-thalassemia patients (less than 4 transfusions per year) who received treatment with HU, were followed prospectively since the beginning of the MIOT network in 2006. For the 43 NTDT patients who started the HU before the 2006, the clinical and instrumental data before the 2006 were retrieved from the MIOT database. The end of the follow-up was in September 2019. Each hematologist completed a case report form detailing patient outcomes between the last available MRI and the end-study date.

Patients were all started in HU therapy at 10 mg/Kg/d, this dosage was then increased by 5 mg/Kg . The choice of the effective dose was achieved following the most favorable balance between the best hematological response and the maximum tolerated dose.

### 2.2. Hematological and biochemical parameters assessment

Blood was collected by venipuncture, allowed to clot, then centrifuged to obtain serum samples. All hematological and biochemical parameters were determined by commercially available kits.

Four different time points were considered for hemoglobin and mean corpuscular volume (MCV) values:

- time 0: before the start of HU treatment,
- time 1: after 6 months of HU treatment,
- time 2: after 12 months of HU treatment,
- time 3: end of the study for the patients who stopped the HU treatment or September 2019 for the patients for whom the treatment was still active at this date.

For all the remaining parameters, the values obtained before the start of HU treatment and after 12 months of therapy were taken into account.

### 2.3. Diagnostic criteria

Pulmonary hyperthension (PHT) was diagnosed if the transtricuspidal velocity jet was greater than 3.2 m/s [12].

The diagnosis of left atrial (LA) enlargement was based on echocardiographic criteria. Indexed LA diameter, four-chamber LA area, and indexed LA volume were categorized according to the joint American and European guidelines [13].

Supraventricular arrhythmias were diagnosed only if ECG-documented and requiring specific medication. Arrhythmias were classified according to the AHA/ACC Guidelines [14].

Leg ulcers were diagnosed according to the presence of diagnostic and clinical signs.

The clinical diagnosis of deep vein thrombosis was confirmed by objective testing using ultrasound or venography.

The diagnosis of extramedullary erythropoiesis (EMH) and fibroadipose involution was made through conventional radiology: computed tomography (CT) or MRI scans.

The cholelithiasis diagnosis was confirmed by noninvasive imaging (ultrasound, MRI or CT).

### 2.4. Statistical analysis

All data were analyzed using SPSS version 17.0 statistical package.

Continuous variables were described as mean ± standard deviation (SD). Categorical variables were expressed as frequencies and percentages.

The normality of distribution of the parameters was assessed by using the Kolmogorov-Smirnov test.

The cumulative incidence was used to assess the absolute risk of cancer over different periods of time. It was estimated using the Kaplan-Meier product-limit method.

One-way repeated measures ANOVA or Friedman test were used to evaluate whether hemoglobin and MCV values were different among the 4 time points and the Bonferroni adjustment was used in all pairwise comparisons. In one-way repeated measures ANOVA the Mauchly's test was used to test assumption of sphericity and when sphericity could not be assumed, Greenhouse-Geisser corrected results were taken.

For categorical variables, the paired comparison pre- vs during-HU treatment was performed using the McNemar's test. For continuous variables, the difference between hematological/biochemical parameters before and after 12 months of HU treatment was analyzed by Student's t-test for paired data or the Wilcoxon signed-rank test.

For continuous values, comparisons between two groups were made by independent-samples t-test or by Mann Withney test. The $\chi 2$ test was used for non-continuous variables.

Correlation analysis was performed using Pearson's or Spearman's test where appropriate.

To determine the best minimum dosage for achieving an increase in hemoglobin levels ≥1.0 g/dl after 12 months, the maximum sum of sensitivity and specificity was calculated from receiver-operating characteristic (ROC) curve analysis.

A 2-tailed probability $P < 0.05$ was considered statistically significant.

### 2.5. Ethical consideration

The study complied with the Declaration of Helsinki and was approved by the institutional review board. All patients gave written informed consent to the protocol.

## 3. Results

### 3.1. Patient data

Table 1 shows the demographic and clinical data of our patients.

Patients started the treatment with HU at a mean age of 37.54 ± 12.68 years (range: 16–70 years) and anemia was the indication in the 88.6% of the cases.

For 7 patients (8.86%) there was a temporary withdraw of the HU treatment, lasted on average 13.57 ± 13.80 months (range: 2–42 months). The causes of the temporary withdraw were: therapy with interferon+ribavirin for hepatitis C virus

Table 1. Demographic and clinical data.

| Variable | All patients (N=79) | Patients treated with HU for more than one year (N=71) |
|---|---|---|
| ***Sex (males/females)*** | 44/35 | 41/30 |
| ***Age of diagnosis (years)*** | 10.21 ± 11.30 | 9.56 ± 9.98 |
| ***Genotype, N (%)*** | | |
| • ***HBB:c.92+6T>C / HBB:c.92+6T>C*** | 14 (17.7) | 10 (14.1) |
| • ***HBB:c.92+6T>C / HBB:c.118C>T*** | 6 (7.6) | 6 (8.5) |
| • ***HBB:c.118C>T / NC_000011.9: g.5241853_5254462del*** | 6 (7.6) | 6 (8.5) |
| • ***HBB:c.118C>T / HBB:c.-137C>G*** | 5 (6.3) | 4 (5.6) |
| • ***HBB:c.93-21G>A / HBB:c.-137C>G*** | 5 (6.3) | 5 (7.0) |
| • ***HBB:c.118C>T / NG_000007.3:g. [63191_70603dup;63291_70703del*** | 3 (3.8) | 3 (4.2) |
| • ***HBB:c.92+6T>C / NC_000011.9: g.5241853_5254462del*** | 3 (3.8) | 3 (4.2) |
| • ***HBB:c.118C>T / HBB:c.118C>T*** | 2 (2.5) | 2 (2.8) |
| • ***HBB:c.315+1G>A / HBB:c.315 +1G>A*** | 2 (2.5) | 2 (2.8) |
| • ***HBB:c.92+6T>C / HBB:c.-137C>G*** | 2 (2.5) | 2 (2.8) |
| • ***HBB:c.-137C>G / NG_000007.3:g. [63191_70603dup;63291_70703- del]*** | 2 (2.5) | 2 (2.8) |
| • ***NG_000007.3:g. [63191_70603dup;63291_70703- del] /nNG_000007.3:g. [63191_70603dup;63291_70703del*** | 2 (2.5) | 2 (2.8) |
| • ***Set of other genotypes present only in one case*** | 21 (26.6) | 18 (25.4) |
| ***Splenectomy before the start of HU treatment, N (%)*** | 65 (82.3) | 58 (81.7) |
| ***Previous history of regular/ occasional transfusions, N (%)*** | 40 (50.6) | 34 (47.9) |
| ***Therapy with folic acid pre-HU, N (%)*** | 15 (19.0) | 14 (19.7) |
| ***Age at the start of the HU treatment (years)*** | 37.59 ± 12.62 | 37.02 ± 12.06 |
| ***Indications for starting HU treatment, N (%)*** | | |
| ***anemia*** | 58 (73.4) | 52 (73.2) |
| ***anemia+EMH*** | 12 (15.2) | 10 (14.1) |
| ***EMH*** | 9 (11.4) | 9 (12.7) |
| ***Mean duration of HU treatment (years)*** | 7.46 ± 5.95 | 8.23 ± 5.79 |
| ***Dose of HU (mg/kg)*** | 15.26 ± 3.55 | 15.56 ± 3.49 |
| ***Occasional transfusions during HU treatment, N (%)*** | 16 (20.3) | 14 (19.7) |
| ***Therapy with folic acid during HU treatment, N (%)*** | 73 (92.4) | 66 (93.0) |

N = number, HU = hydroxyurea, EMH = extramedullary erythropoiesis.

infection in two cases, ulcers in two cases, reduction in efficacy in one case, interaction with another drug in one case, and cerebral vasculitis in one patient.

Mean duration of HU treatment was 7.46 ± 5.95 years (range 0.4–22 years).

### 3.2. Treatment interruption and adverse events

Forty-five (57.0%) patients had stopped the treatment with HU before September 2019, due to a decrease in efficacy in the 44.4% (N = 20) of the cases. The other causes for the interruption of HU treatment were: patient choice (N = 6), death (N = 3), luspatercept screening (N = 3), ulcers (N = 2), cancer (N = 2), asthenia (N = 2), leukopenia (N = 2), pulmonary hypertension (N = 1), extrasystoles (N = 1), pregnancy desire (N = 1), antiviral therapy (N = 1), splenectomy (N = 1). Compared to the 34 patients for whom the therapy was still active in September 2019, these patients showed a significantly lower mean duration of the treatment (4.99 ± 4.11 years vs 10.71 ± 6.47 years; P < 0.0001).

Three (3.8%) patients died during the HU treatment, one patient due to heart failure, one due to respiratory failure, and one due to sepsis.

Two (2.5%) patients had a tumor during the treatment with HU: one hepatocarcinoma after 2 year of treatment and one acute leukemia after 13 years of treatment. The cumulative incidence of tumors was 0.0 after 1 year of treatment, 1.64 after 5 years of treatment, 1.64 after 10 years of treatment, and 6.82 after 15 years of treatment.

There were 5 (6.3%) adverse events: 3 leg ulcers and 2 leukopenias. The incidence of adverse events was 0.85/100 patient/year of treatment.

For eight patients the duration of HU treatment was <12 months. In seven patients there was a precocious interruption due to inefficacy (N = 3), thrombocytopenia/leukopenia (N = 2), patient choice (N = 1) or death (N = 1). In one patient the treatment was still on course in September 2019, but it was started in November 2018.

All following analyses were focused on the 71 patients treated for more than 12 months (Table 1). The mean follow-up of these patients, that is the duration of HU treatment, was 8.23 ± 5.79 years (range: 1.1–22 years).

### 3.3. Changes in hemoglobin and MCV values during the treatment with HU

Figure 1a shows the hemoglobin values at the 4 different time points; a significant difference was detected (P < 0.0001). The hemoglobin values at time 0 were significantly lower than the values registered at time 1 and at time 2 (P = 0.014 and P = 0.008, respectively), but were comparable to the values registered at time 3. The hemoglobin values at time 1 as well as at time 2 were significantly higher than values at time 3 (P = 0.022 and P = 0.001, respectively).

Figure 1b shows the MCV values at the 4 different time points and a significant difference was detected (P < 0.0001). The MCV at time 0 was significantly lower than the MCV at time 1 (P < 0.0001), at time 2 (P < 0.0001), and at time 3 (P < 0.0001)

### 3.4. HU treatment and symptoms, instrumental parameters, and complications

During HU treatment we did not detect significant changes in the frequency of dyspnea, asthenia, atrial dilatations, cardiovascular complications (pulmonary hypertension, arrhythmias, ulcers, and deep vein thrombosis), EMH, and fibroadipose involution of the EMH masses (Table 2). Five patients underwent cholecystectomy before the start of the HU therapy and,

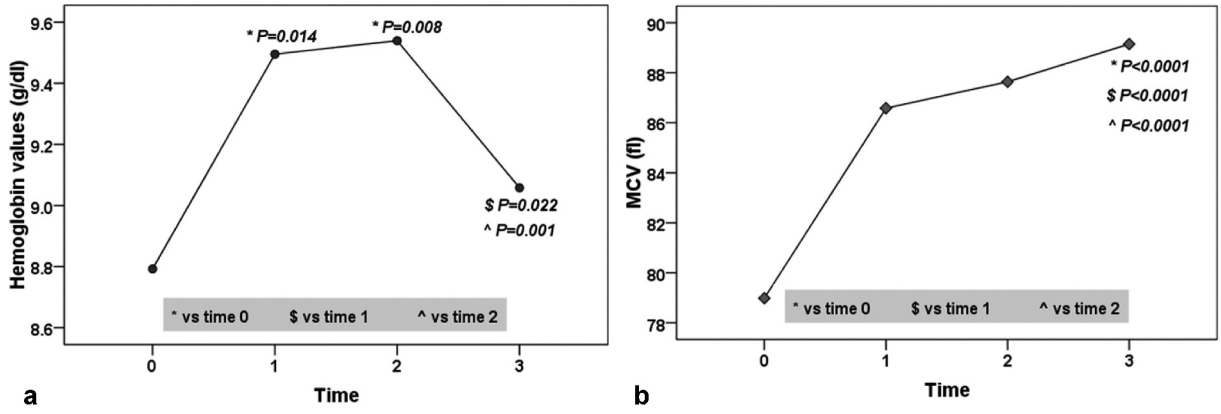


Figure 1. Hemoglobin values (a) and MCV (b) at the 4 different time points. Time 0 = before the start of HU treatment; time 1 = after 6 months of HU treatment; time 2 = after 12 months of HU treatment; time 3 = end of the study for the patients who stopped the HU treatment or September 2019 for the patients for whom the treatment was still active at this date.

Table 2. Frequency of different complications before the start and during the HU therapy. The new occurrences and the remissions after the start of the HU therapy are indicated.

| | | Comparison between pre-HU and under HU treatment | | | | |
|---|---|---|---|---|---|---|
| Complication | N | Before HU therapyN (%) | During HU therapyN (%) | P | Changes in patients with the complication occurred before the start of HU treatment(N) | New occurrences(N) |
| ***Dyspnea*** | 70 | 15 (21.4) | 16 (22.9) | 1.000 | 1 remission<br>9 improvement<br>4 stable<br>1 worsening | 2 |
| ***Asthenia*** | 70 | 29 (41.4) | 29 (41.4) | 1.000 | 2 remission<br>17 improvement<br>10 stable | 2 |
| ***Pulmonary hyperthension*** | 69 | 6 (8.7) | 8 (11.6) | 0.500 | 3 improvement<br>3 stable | 2 |
| ***Atrial dilatation*** | 68 | 20 (29.4) | 20 (29.4) | 1.000 | 1 remission<br>4 improvement<br>13 stable<br>2 worsening | 1 |
| ***Atrial arrhythmias*** | 70 | 5 (7.1) | 5 (7.1) | 1.000 | 1 remission<br>4 stable | 1 |
| ***Leg ulcers*** | 70 | 14 (20.0) | 16 (22.9) | 0.625 | 1 remission<br>6 improvement<br>6 stable<br>1 worsening | 3 |
| ***Deep vein thrombosis*** | 69 | 3 (4.3) | 7 (10.1) | 0.219 | 1 remission1 stable1 worsening | 5 |
| ***Extramedullary erythropoiesis*** | 59 | 24 (40.7) | 24 (40.7) | 1.000 | 1 remission<br>18 improvement<br>3 stable<br>2 worsening | 1 |
| ***Fibroadipose involution*** | 52 | 5 (9.6) | 10 (19.2) | 0.125 | 1 remission<br>4 stable | 6 |
| ***Cholelithiasis in patients without cholecystectomy*** | 59 | 13 (22.0) | 19 (32.2) | 0.031 | 13 stable | 6 |

N = number, HU = hydroxyurea.

considering only the patients who never had a cholecystectomy, the frequency of cholelithiasis significantly increased after the start of HU therapy.

### 3.5. Changes in hematological/biochemical parameters after 12 months of treatment

After 12 months of therapy a significant increase in hemoglobin and MCV values and a significant decrease in the number of white blood cells (WBC), indirect bilirubin, nucleated red blood cells (NRBC), and platelets, in uric acid levels, and in soluble transferrin receptor values were detected (Table 3).

### 3.6. Comparison between responders and non-responders

For 63 patients hemoglobin values at baseline (before the start of HU treatment) as well as after 12 months of treatment were available. After 12 months of treatment a decrease in hemoglobin values was detected in 11 (17.5%) patients, an increase <1 g/dl in 24 (38.1%) patients, an increase ≥1 g/dl and <2 g/dl

Table 3. Hematological and biochemical parameters before and after 12 months of HU treatment.

| | N | Before HU treatment | After 12 months of HU treatment | P |
|---|---|---|---|---|
| ***Hemoglobin (g/dl)*** | 63 | 8.42 ± 1.06 | 9.39 ± 1.17 | <0.0001 |
| ***MCV (fl)*** | 55 | 76.52 ± 12.27 | 86.39 ± 11.49 | <0.0001 |
| ***Hemoglobin F (%)*** | 50 | 52.58 ± 27.02 | 62.88 ± 26.14 | <0.0001 |
| ***Reticulocytes (%)*** | 12 | 29.17 ± 39.53 | 32.08 ± 40.20 | 0.480 |
| ***WBC (cells/mm$^3$)*** | 58 | 18314.83 ± 17241.48 | 13212.89 ± 12369.15 | 0.001 |
| ***NRBC (%)*** | 26 | 78.92 ± 129.86 | 63.23 ± 69.98 | 0.040 |
| ***Platelets (10$^3$/mm$^3$)*** | 59 | 651.73 ± 296.93 | 565.17 ± 253.34 | <0.0001 |
| ***LDH (u/l)*** | 34 | 537.50 ± 192.89 | 512.26 ± 160.44 | 0.326 |
| ***Indirect bilirubin (mg/dl)*** | 40 | 2.57 ± 1.56 | 2.17 ± 1.55 | 0.002 |
| ***Uric acid (mg/dl)*** | 42 | 5.85 ± 1.37 | 5.22 ± 1.32 | 0.026 |
| ***Serum ferritin (ng/ml)*** | 42 | 474.99 ± 343.81 | 438.74 ± 460.57 | 0.165 |
| ***Soluble transferrin receptor (mg/l)*** | 12 | 10.41 ± 1.99 | 7.62 ± 2.50 | 0.007 |

N = number, HU = hydroxyurea, MCV = mean corpuscular volume; WBC = white blood cells, NRBC = nucleated red blood cells, LDH = lactate dehydrogenase.

in 17 (27.0) patients, and an increase ≥2 g/dl in 11 (17.5%) patients.

The change in hemoglobin values after 12 months of therapy showed a significant positive correlation with the dose of HU (R = 0.441; P < 0.0001) and a significant inverse correlation with the baseline hemoglobin values (R = −0.459; P < 0.0001).

We defined responders the 28 (44.4%) patients who achieved a ≥ 1.0 g/dl increase in hemoglobin levels after 12 months (mean increase 1.95 ± 0.97 g/dl). Table 4 shows the comparison between responders and non-responders. Responders received a significant higher dose of HU and showed significantly lower pre-treatment hemoglobin values. Using ROC curve analysis, a dosage greater than 16.6 mg/dl discriminated the responders with acceptable sensitivity and good specificity (59.3% and 85.7%, respectively) and with an area under the curve (AUC) of 0.75 (P < 0.0001).

The responders experienced a significantly higher decrease in white blood cells after 12 months of treatment.

## 4. Discussion

To our knowledge, this study is the first trying to perform a balance in terms of clinical efficacy and safety on HU administration in adult patients with NTDT. Anemia and extramedullary hematopoiesis were the most common indications for receiving HU and most of the patients started the treatment when they were about 40 years old. It likely reflects the well-documented absence of EMH in younger patients with NTDT [15,16], the current uncertainty about of long-term use of HU, and the impact on fertility in childhood.

We followed our NTDT patients in HU treatment for 10.71 ± 6.47 years, and in our knowledge, it represents the

Table 4. Comparison between responders and non-responders.

| | Non-Responders (N = 35) | Responders (N = 28) | P-value |
|---|---|---|---|
| ***Female Sex, N (%)*** | 16 (45.7) | 11 (39.3) | 0.798 |
| ***Age at the start of the treatment (yrs)*** | 39.78 ± 14.01 | 33.31 ± 10.86 | 0.077 |
| ***Dose of HU (mg/kg)*** | 14.22 ± 3.05 | 17.22 ± 3.39 | 0.001 |
| ***Splenectomy before the start of HU treatment, N (%)*** | 27 (77.1) | 23 (82.1) | 0.758 |
| Hematological and biochemical parameters before HU treatment | | | |
| ***Hemoglobin (g/dl)*** | 8.75 ± 0.91 | 8.00 ± 1.11 | 0.004 |
| ***MCV (fl)*** | 76.35 ± 15.38 | 76.23 ± 7.97 | 0.658 |
| ***Hemoglobin F (%)*** | 46.72 ± 28.66 | 59.22 ± 22.60 | 0.074 |
| ***Reticulocytes (%)*** | 38.99 ± 45.16 | 29.52 ± 34.98 | 0.643 |
| ***WBC (cells/mm$^3$)*** | 15,627.06 ± 10,345.98 | 22,613.08 ± 22,836.83 | 0.146 |
| ***NRBC (%)*** | 90.06 ± 154.57 | 64.21 ± 56.37 | 0.740 |
| ***Platelets (10$^3$/mm$^3$)*** | 609.35 ± 279.57 | 697.04 ± 306.58 | 0.264 |
| ***LDH (u/l)*** | 546.54 ± 219.91 | 526.73 ± 161.56 | 0.702 |
| ***Indirect bilirubin (mg/dl)*** | 2.52 ± 1.66 | 2.75 ± 1.37 | 0.346 |
| ***Uric acid (mg/dl)*** | 5.70 ± 1.24 | 6.02 ± 1.68 | 0.476 |
| ***Serum ferritin (ng/ml)*** | 427.05 ± 269.63 | 673.80 ± 533.07 | 0.033 |
| Changes in hematological and biochemical parameters after 12 months of treatment | | | |
| ***Mean difference in hemoglobin (g/dl)*** | 0.20 ± 0.59 | 1.95 ± 0.97 | <0.0001 |
| ***Mean difference in MCV (fl)*** | 8.72 ± 16.68 | 11.16 ± 9.82 | 0.376 |
| ***Mean difference in hemoglobin F (%)*** | 7.49 ± 19.36 | 13.35 ± 12.36 | 0.080 |
| ***Mean difference in reticulocytes (%)*** | 15.32 ± 26.46 | −9.49 ± 31.56 | 0.171 |
| ***Mean difference in WBC (cells/mm$^3$)*** | −1925.39 ± 6230.29 | −9294.96 ± 22,146.35 | 0.022 |
| ***Mean difference in NRBC (%)*** | −28.13 ± 87.71 | 4.22 ± 46.51 | 0.598 |
| ***Mean difference in platelets (10$^3$/mm$^3$)*** | −69.00 ± 216.97 | −108.85 ± 209.75 | 0.703 |
| ***Mean difference in LDH (u/l)*** | 0.50 ± 203.13 | −87.00 ± 54.04 | 0.061 |
| ***Mean difference in indirect bilirubin (mg/dl)*** | −0.19 ± 0.99 | −0.83 ± 0.98 | 0.174 |
| ***Mean difference in uric acid (mg/dl)*** | −0.50 ± 1.74 | −0.92 ± 1.48 | 0.460 |
| ***Mean difference in serum ferritin (ng/ml)*** | −36.30 ± 169.01 | −36.14 ± 344.93 | 0.350 |

N = number, HU = hydroxyurea, MCV = mean corpuscular volume; WBC = white blood cells, NRBC = nucleated red blood cells, LDH = lactate dehydrogenase.

longest follow-up for a group of NTDT adult patients under HU [17]. We confirmed that the decrease in efficacy was a major cause of treatment interruption.

Mortality was noteworthy (3.8%), but probably well-suited with the life expectancy observed in adult cohorts of NTDT patients [18]. The linkage between HU administration and cancer occurrence has been always controversy also in chronic myeloproliferative disorders [19]. On the other hand, it is well recognized that patients with thalassemia are at higher risk of cancer than general population [20]. We observed two cases of tumors, but for the case of hepatocellular carcinoma (HCC), the cumulative incidence was comparable with that previously reported among NTDT patients in a large series [21]. Furthermore, HCC is an emerging and growing entity among patients with thalassemia which have well-recognized risk factors for developing HCC independently from the HU treatment [22]. Conversely, for the observed case of leukemia, comparable data are not available, but in patients with TDT and NTDT several cases of hematologic malignancies have been previously described. A recent update in this field reported not only the first cases of monoclonal gammopathy of undetermined significant (MGUS), but also a 45-year-old female with HbE disease, not treated with HU therapy who developed acute myeloid leukemia [23]. Overall, our observations may not support a carcinogenic potential for the HU in the NTDT patients due to the so long exposure and the intrinsic but well-defined spontaneous rate for cancer development in NTDT; anyway, further studies are needed to better assess any potential relationship.

Side effects requiring the withdraw of the therapy were those commonly described in other series, but cutaneous alteration/cancer and neurological toxicity were not reported in our population.

The efficacy of HU was assessed in the 71 patients who continued the treatment and did not drop out, mainly because they were not responding to the treatment. After 12 months of treatment, HU induced not only a significant increase in MCV and Hb, but also a down-regulation of all erythropoietic and/or hemolysis indices, as previously demonstrated [24–27]. However, at final evaluation, the loss of statistical significance in the increase in Hb but not in the MCV value was observed, confirming that macrocytosis is not only a well known, but also long-lasting side effect of hydroxyurea.

Regarding the explored complications, they were generally comparable in terms of prevalence to other series [16,28,29]. No so many new occurrences or remissions were observed, and no statistically significant difference was found for each complication comparing between the pre-HU and the under-HU time intervals, but improvement and/or stabilization in several complications and/or symptoms were retrieved. Particularly, out of the 24 cases of EMH reported, 18 ameliorated. So, HU treatment may have a broad favorable impact in limiting EMH considering the long follow-up and the natural progressivity of this complication with aging, particularly during the 4–5$^{th}$ decades [15]. Generally, these data are in agreement with the 'Optimal care' study, where HU treatment was associated with an increased risk of hypogonadism, but it was protective for the development of EMH, PHT, leg ulcers, hypothyroidism, and osteoporosis [30]. At the best of our knowledge, there are no reports of direct cardiotoxicity in patients with thalassemia assuming hydroxyurea. The new occurrence of cardiac events reported in our series (pulmonary hypertension and atrial fibrillation) are generally comparable in terms of prevalence to other series and in first hypothesis they seem to be ascribed to the natural and well described progression of the disease.

In our patients the hematological response rate was around 45% at indicated doses, but it dropped and loose of statistical significance in long-term treatment. However, data on complications were probably so convincing that in the real life most clinicians decided to maintain several patients under HU treatment, despite the absence of a concomitant optimal long-term hematological response.

Interestingly, at baseline the 28 responder patients showed significantly lower Hb levels and higher ferritin levels in comparison to the no-responder patients, thus suggesting the former had a more severe form of NTDT. No 'hematological responder genotype' was found in our series; however, the responder patients showed the tendency to have higher baseline value of HbF and higher increase in HbF following HU treatment. HbF level is correlated with polymorphisms such as the XmnI (Gγ) SNP (−158C>T) [31], commonly associated to HU-response. Therefore, our data appeared to be consistent with those reported recently 'in vitro' and based on the proliferation, differentiation kinetics and gene expression profiles of erythroblasts, showing that basal HbF level could be a fundamental determinant of HU responsiveness [32].

### 4.1. Limitations

This is a retrospective study with several patients lost to follow up and without appropriate measurements of compliance to the HU treatment.

In order to limit the heterogeneity of the NTDT population, we evaluated only the milder forms (no or < 4 transfusions per year) which do not represent all the spectrum of this disease.

Just over half of the enrolled patients started the HU before the beginning of the MIOT network and the clinical and instrumental data before the 2006 were retrieved from the MIOT database.

Due to its observational nature, the study lacked a control population (age- and sex-matched) to compare the natural progression in complications of the disease and a potential decrease in hemoglobin level over the long observation period of the study without the use of HU.

The criterion applied for the identification of the responders was arbitrary and not validated. However, it has been recently shown that an improvement in Hb levels by 1 g/dL could decrease the risk of developing morbidities in patients with NTDT whose baseline Hb was less than 10 g/dL [33].

## 5. Conclusion

This study seems to suggest that not only for the hematological responder patients, but also for those affected by specific complications, HU could be still a valid option to limit the advance of the overall clinical burden disease without carrying significant adverse events and increase in mortality.

Limited treatment options such as the start of regular blood transfusions (BT) are available for managing the disease clinical burden in adult patients with NTDT [34,35]. However, despite regular BT may represent a way to avoid the natural progression of the disease [34], they are associated with complications such as iron overload and allo/autoimmunization. Positive data on the effects on Hb values of Luspatercept (ACE-536) are challenging [36]. Results from the BEYOND study further confirm the clinical potential of Luspatercept to maintain the elevation of hemoglobin levels in a large series of NTDT patients regardless of their baseline hemoglobin status [37]. However, in the NTDT patients randomized prospective clinical trials designed to investigate the role of various interventions on the full spectrum of the complications are needed.

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## Declaration of interest

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## Author's contribution

P Ricchi conceived and designed the study and drafted the initial manuscript. A Meloni analyzed the data and drafted the initial manuscript. P Rigano contributed to the design of the study and collected the data. L Pistoia was responsible for data collection. A Spasiano, M Allò, G Messina, A Quarta, R Rosso, A Quota, A Filosa, and A Maggio collected the data. A Pepe supervised the study and she is the guarantor of this work. All authors assisted with interpretation, commented on drafts of the manuscript, and approved the final version.

## Data availability

The data that support the findings of this study are available on request from the corresponding author, A Pepe.

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