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Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine

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A B S T R A C T

Background and Objectives. Seven Italian centers reported data on survival, causes of death and appearance of complications in patients with thalassemia major. The interactions between gender, birth cohort, complications, and ferritin on survival and complications were analyzed.

Design and Methods. Survival after the first decade was studied for 977 patients born since 1960 whereas survival since birth and complication appearance was studied for the 720 patients born after 1970. Better survival was demonstrated for patients born in more recent years (p<0.00005) and for females (p=0.0003); 68% of the patients are alive at the age of 35 years. In the entire population 67% of the deaths were due to heart disease.

Results. There was a significant association between birth cohort and complication-free survival (p<0.0005). The prevalence of complications was: heart failure 6.8%, arrhythmia 5.7%, hypogonadism 54.7%, hypothyroidism 10.8%, diabetes 6.4%, HIV infection 1.7%, and thrombosis 1.1%. Lower ferritin levels were associated with a lower probability of heart failure (hazard ratio =3.35, p<0.005) and with prolonged survival (hazard ratio = 2.45, p<0.005), using a cut-off as low as 1,000 ng/mL.

Interpretation and Conclusions. Survival and complication-free survival of patients with thalassemia major continue to improve, especially for female patients born shortly before or after the availability of iron chelation.

Key words: thalassemia, survival, causes of death, ferritin, hemosiderosis.

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Thalassemia has traditionally been considered a pediatric disease, not only because it becomes symptomatic in the first few months of life, but also because, in the past, most patients died before adolescence. In the late 1970s, 50% of Italian thalassemic patients died before the age of 12 years old.[1] and patients followed between 1960 and 1976 at Cornell Medical Center had a median survival of 17.1 years.[2] Regular blood transfusions and iron chelation with deferoxamine have changed the prognosis of the disease. Bone marrow transplantation, so far the only definitive cure for thalassemia, became available in 1981[3] and technological advances continue to be reported.[4] Therefore, reliable data on the prognosis of patients treated conventionally have become of paramount importance in order to make meaningful decisions regarding genetic counseling and choice of therapy for children who have an HLA-matched donor. In 1989 we reported on the survival of a large population of Italian patients,[5] which was later updated,[6] but no data were available on the prevalence of the most frequent complications that are known to affect thalassemia. The quantitative evaluation of iron overload is also difficult and the role of serum ferritin is debated. In this report we present the patients' survival data eleven years later, together with the frequency of seven major complications, and the associations between survival, gender, complications, and ferritin levels.

Design and Methods

Seven Italian teaching hospitals contributed data for this study starting in 1983. Patients included were born on or after January 1, 1960 and had been diagnosed as being affected by thalassemia major (transfusion-dependent) before the age of 3 years. Since all the records were complete and reli-

able for all centers as of 1970, survival after the age of 10 was calculated for the entire population, while survival and complication-free survival since birth were analyzed separately for patients born in 1970 or later. All the patients had an Italian background and were uniformly treated. Until 1970, patients were transfused when hemoglobin (Hb) reached a level of 6-7 g/dL; later, pre--transfusional Hb was maintained at 9-9.5 g/dL, with the exception of a period of six years (1981-1986) when it was kept at approximately 11 g/dL. Deferoxamine was introduced in 1975 as an intramuscular injection and in 1980 as a subcutaneous infusion (40-50 mg/kg). Since 1995, 121 patients included in the study were switched to the oral chelator deferiprone, either because they entered a research protocol or because of lack of compliance with, or toxicity to, desferrioxamine. The median therapy time was 3.09 years, the range was from 1 week to 4.85 years. These patients were censored at the time they started deferiprone therapy. No patient was treated with combination therapy. Patients underwent splenectomy if the volume of blood transfused exceeded 250 mg/kg/year. The data collected included: date of birth, date of diagnosis, date of death, cause of death, time of appearance of insulin-dependent diabetes, hypothyroidism requiring replacement therapy (i.e. in the presence of low to marginally low free thyroxine and abnormally high levels of thyroid-stimulating hormone),[7] heart failure and arrhythmia requiring inotropic or anti-arrhythmic therapy, thrombosis, HIV infection, and hypogonadism, defined as the absence of breast development by the age of 15 in females and the absence of testicular enlargement by 17 years old in boys. The last follow-up was 31 December, 1999.

Mean yearly serum ferritin levels, based on a minimum of three measurements per year, were obtained for a subgroup of patients starting in 1991. Average ferritin values for each patient before any complication occurred were used to examine the relationships between complications and ferritin levels.

Statistical Methods

The Kaplan-Meier method was used to estimate survival and complication-free survival. For complication-free survival analysis, time to first complication was used as the failure event. When evaluating complication-free survival, lack of sexual maturation was not considered as a complication.[8]

The survival and complication-free survival curves were compared by gender and by 5-year birth cohorts starting from 1960, using the log-rank test. Cox regression models with time-varying covariates were used to explore associations between time of complications and survival time. Complications first observed at death were not included since they had no ability to predict survival. Cox regression models with multiple types of complications were also fitted using a *best-subset* approach.[9] The best fitting model was chosen based on the criterion that further addition of predictors did not significantly increase the log likelihood. Patients who underwent bone-marrow transplantation were censored for all analyses at the time of the procedure.

χ^2 and Wilcoxon's tests were used to examine the possible associations between gender and complications, as well as to compare birth cohort, gender, complications, age, and survival between patients for whom ferritin levels were available and those for whom they were not available, in order to explore whether the patients without ferritin data were any different from those with ferritin data included in the analysis. The relationship between survival and ferritin levels was performed using Cox regression with time-varying covariates. Ferritin values, averaged over a year, were assumed to be taken at mid-year.

A statistically significant difference was defined at $p<0.05$. Analyses were performed using Stata 7.0 for Windows software (2001).[10]

Results

Overall analysis

The overall analysis population was formed of 1,073 patients, 501 (46.7%) of whom were females and 572 males. Figure 1 reports the subgroups analyzed in this report.

The median age at diagnosis was 7 months (range 1 day to 3 years). Sex distribution was similar in the different birth cohorts. One hundred and five patients (51% males) underwent bone marrow transplantation and were censored at the time of transplant.

Of the 1,073 patients, 977 survived beyond the first decade of life and were available for Kaplan-Meier analysis. Figure 2A shows improved survival for patients born in more recent years (log-rank test $p<0.00005$). The better overall survival of females is shown in Figure 2B ($p=0.0003$).

Patients in more recent cohorts were less likely to die from cardiac disease (log rank test, $p<0.00005$) (Figure 3). Only one patient died from this complication in the 1980-1984 birth cohort and there were no deaths from heart disease afterwards. The causes of death are listed in Table 1.

Survival from birth and complication free-survival

Data were available for 720 patients born after 1970. Survival from birth according to birth cohort is illustrated in Figure 4A. Survival improved in later cohorts ($p=0.024$), as it did in the entire population. In addition,

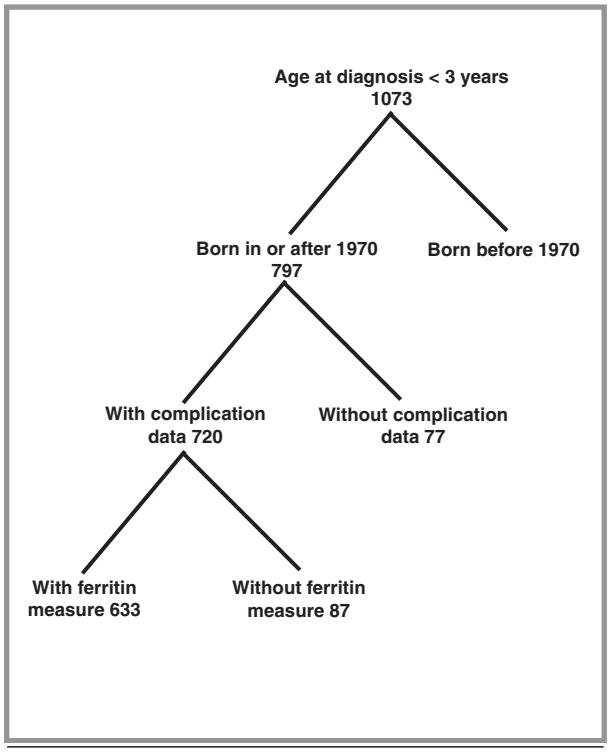

Figure 1 Flow-chart describing the subgroups of the study population.

Table 1. Causes of death for the entire population of patients and for those born after 1970.

	All patients (N=1073)		*Patients born after 1970 (N=720)*	
	N	*%*	*N*	*%*
Heart Failure	133	60.2	31	50.8
Infection	15	6.8	9	14.8
Arrhythmia	15	6.8	4	6.6
Myocardial infarction	4	1.8		
Cirrhosis	9	4.1		
Thrombosis	9	4.1	2	3.3
Malignancy	8	3.6	2	3.3
Diabetes	7	3.2	2	3.3
Accident	4	1.8	1	1.6
Renal Failure	3	1.4		
HIV/AIDS	3	1.4	2	3.3
Familial autoimmune disorder	2	0.9	1	1.6
Anorexia	1	0.5	1	1.6
Hemolytic anemia	1	0.5	1	1.6
Thrombocytopenia	1	0.5		
Unknown	6	2.7	5	8.2
Total	221		61	

females were, again, found to have a better survival than males (survival at age 25 years 92.2% vs. 83.5%, p=0.0055). Complications for this group of patients are listed in Table 2. One hundred and fifty-seven patients (21.8%) developed at least one complication.

If patients had more than one complication, only the time to the first complication was considered. Median age at the appearance of the first complication was 16 years, the range was from 5.4 to 28.4 years. Figure 4B illustrates complication-free survival by birth cohort. Two hundred and fifty-one males and 248 females reached the age of 17 and 15, respectively, and were assessed for puberty. Of these, 137 (54.6%) males and 136 (54.8%) females did not show pubertal maturation. One hundred and sixteen males and 112 females received replacement hormonal therapy. The percentage of patients affected by hypogonadism was not different among those born in 1970-74 and those born in 1975-79 (64.5% and 56.3%, respectively), but was much lower among those in the birth cohort 1980-84 (14.3%). The percentage of patients who developed diabetes and hypothyroidism was also lower in the more

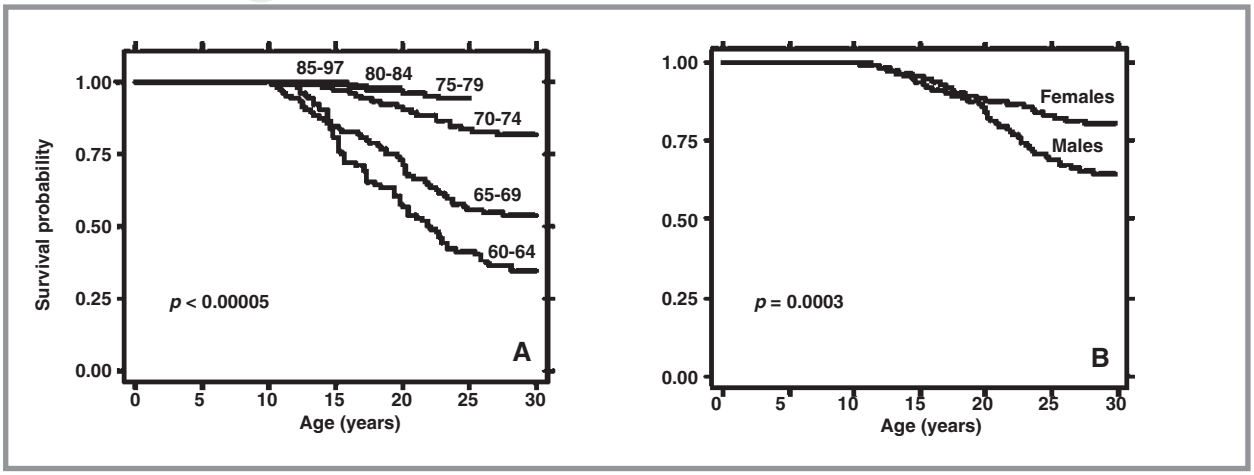

Figure 2. Kaplan-Meier survival curves, after the first decade of life, by birth cohort (A) and by sex (B).

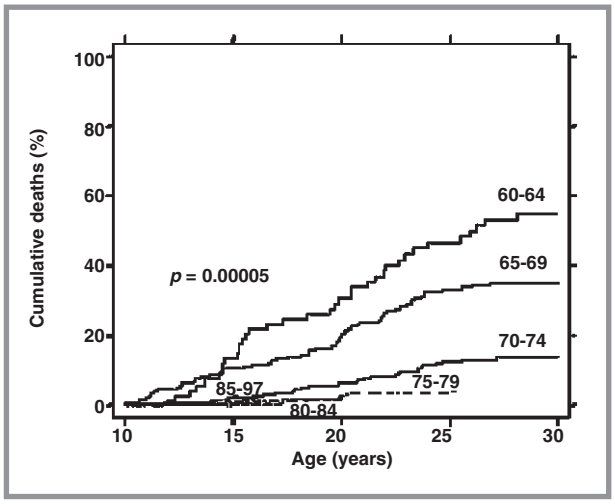

Figure 3. Probability of cardiac death (heart failure and arrhythmia) by cohort of birth. The Kaplan-Meier estimates for the probability of death from heart disease at age 20 were 2.5%, 6.3%, 20%, and 30% for the 75-79, 70-74, 65-69, and 60-64 birth cohorts, respectively.

Table 2. Distribution of complications affecting 720 patients born after 1970.

Complication	*N*	*%*
HEART FAILURE	49	6.8
ARRHYTHMIA	41	5.7
DIABETES	46	6.4
HIV INFECTION	12	1.7
THROMBOSIS	8	1.1
HYPOTHYROIDISM	78	10.8
HYPOGONADISM*	273	54.7

**Only 499 patients were old enough to be assessed for hypogonadism.*

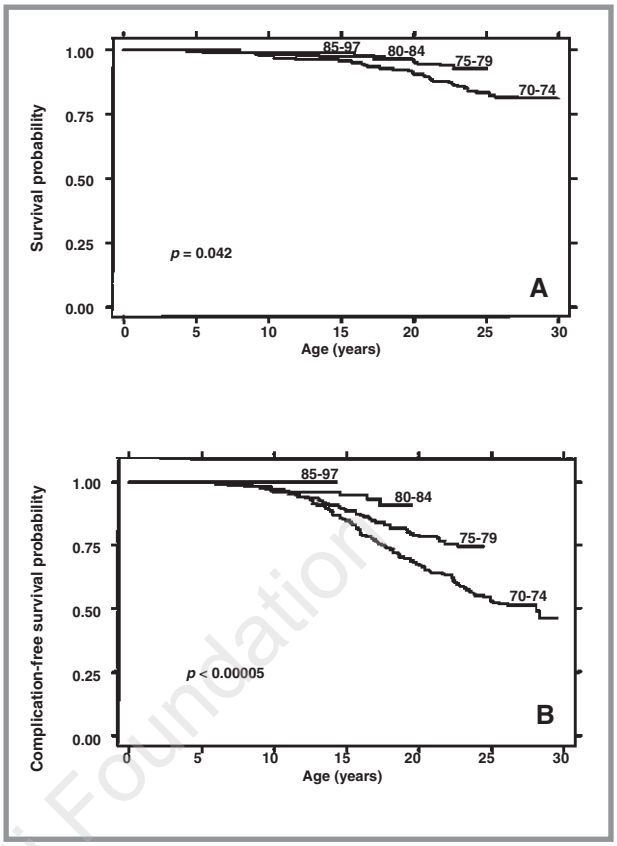

Figure 4. A. Survival curves, from birth, of patients born after 1970, subdivided by birth cohort. B. Complication-free survival by cohort of birth. Patients born in later years had better complication-free survival, and developed the first complication later, although estimation beyond 15 years is not available for the cohort born in 1985 or later. Hypogonadism is not accounted for in the curves.

recent cohorts (15.5% for 1970-74, 2.5% for 1975-79 and 0.8% for 1980-84, 0 afterwards, for diabetes, p <0.0005; and 16.7%, 12.3%, 4.9% and 0, for hypothyroidism, p<0.0005). If only patients born prior to 1980 were considered, there was still a significant association between diabetes (p<0.0005), but not hypothyroidism, and birth cohort.

There was an association between sex and heart failure (p=0.0063). Male patients were more likely to develop heart failure than were female patients. Of the 49 patients with heart failure 35 (71.4%) were males. The median age at heart failure was 16.6 (range 7–25) years. Table 3 shows the association between complications, age, and overall survival for prognostic complication, using a Cox regression model with a time-varying covariate. On the basis of this univariate analysis, sex, heart failure, arrhythmia, diabetes, and hypothyroidism were significant factors for predicting survival. Human immunodeficiency virus (HIV) infection and thrombosis were too infrequent to be predictive. When combining these to a single model to include multiple complications, heart failure, was prognostic in the presence of other prognostic variables. Arrhythmia remained prognostic in the presence of heart failure, and the best-fitting model included both gender and diabetes; hypothyroidism, while prognostic when considered separately, did not add predictive power above heart failure as a prognostic variable.

Cox regression models were fitted for a subpopulation of 633 patients using ferritin as a time-varying covariate. There were 50 deaths in this group. Ferritin was used as a continuous (on a log scale) as well as dichotomized variable at various cut-off levels. Lower ferritin was associated with prolonged survival (log scale, hazard ratio 2.67, p<0.0005). Ferritin was prognostic at a cut-off of 2,500 ng/mL (hazard ratio 4.43, p<0.0005) and remained prognostic after using a cut-off as low as 1,000 ng/mL to define high and low fer-

Table 3. Summary of overall survival based on separate Cox regression models using complications as time-varying covariates (720 subjects, 62 deaths).

Factor	*p value*	*Hazard ratio (95% CI)*
Age at diagnosis	0.37	1.07 (0.74 – 1.55)
Sex (M vs. F)	0.007	2.14 (1.23 – 3.71)
Heart failure (Yes vs. No)	< 0.0005	25.6 (14.3 – 45.6)
Arrhythmia (Yes vs. No)	< 0.0005	17.2 (9.2 – 32.3)
Diabetes (Yes vs. No)	0.009	2.83 (1.3 – 6.15)
Hypothyroidism (Yes vs. No)	0.009	2.42 (1.24 – 4.71)

The hazard ratio shows the relative risk of death of patients in the first category relative to the second except for age; CI= confidence interval.

Table 4. Summary of overall survival based on multiple variable Cox regression models using ferritin and heart disease (complications) as time-varying covariates (633 subjects, 50 deaths).

Model	*Factor*	*p value*	*Hazard ratio (95% CI)*
A	Ferritin (log)	< 0.0005	2.37 (1.54 – 3.64)
	Heart failure (Yes vs. No)	< 0.0005	10.2 (4.71 – 22.3)
	Arrhythmia (Yes vs. No)	0.041	2.49 (1.09– 5.66)
	Sex (M vs. F)	0.03	1.98 (1.03 – 3.83)
B	Ferritin (>2500 vs. < 2500)	< 0.0005	3.65 (2.04 – 6.54)
	Heart failure (Yes vs. No)	< 0.0005	11.3 (5.25 – 24.2)
	Arrhythmia (Yes vs. No)	0.04	2.35 (1.04 – 5.32)
	Sex (M vs. F)	0.05	1.93 (1.00 – 3.71)

ritin levels (hazard ratio 2.61, p=0.029). Among the patients who died, the last ferritin level was higher than 2,500 ng/mL in 30 (60%) and higher than 1,000 ng/mL in 44 (88%). Various models in which ferritin levels, gender and complications were used to predict survival were fitted, following the algorithm outlined in the methods section. The two best-fitting models included ferritin, heart failure, arrhythmia and sex, in which ferritin was either on the log scale, or used with a cut-off point of 2,500 ng/mL. Details are shown in Table 4.

Six hundred and twenty-one patients were available for Cox regression models to assess the relationship between ferritin and heart failure using ferritin as the time-varying covariate. There were 41 cases of heart failure. Patients with lower ferritin were less likely to have heart failure (log scale, hazard ratio 3.4, p<0.0005). Ferritin was prognostic at a cutoff of 2,500 ng/mL (hazard ratio 4.82, p<0.0005) and remained prognostic after using a cut-off as low as 1,000 ng/mL to define high and low ferritin levels (hazard ratio 3.21, p=0.028). The relationship between heart failure and ferritin remained, even after controlling for gender.

Two hundred and ninety-six patients were available to assess the relationship between ferritin and hypogonadism: 94 were hypogonadic. Lower ferritin levels were marginally associated with a lower probability of hypogonadism (log scale, hazard ratio 1.31, p=0.059). Ferritin was not prognostic at a cut-off of either 2,500 ng/mL or 1,000 ng/mL.

Ferritin level did not appear to be a significant predictor of time to hypothyroidism. The event rates (11/602) for diabetes were too low to fit a Cox model.

Discussion

In 1989 we reported on survival in a large group of patients with thalassemia major receiving conventional therapy. The present study reports follow-up data, 11 years later,[5] on survival and causes of death of the largest population of uniformly treated patients with transfusion-dependent thalassemia described so far, and provides new data on complications and on the predictive value of serum ferritin, as a measure of iron overload, in a subgroup of the same patients.

Transfusion-dependent patients, in the absence of chelation therapy, develop progressive accumulation of iron, which is responsible for tissue damage and, eventually, death. The role of transfusion and chelation in improving survival of thalassemia patients has been reported.[2,5,11,12] In a recent study from Great Britain it was found that 50% of patients died before reaching the age of 35[13] and the survival curve of the cohort born in 1975-84 resembled that of the cohorts born in 1945-54 and 1955-64. In our population 68% of the patients were still alive at the age of 35 years and we observed a constant improvement in survival for patients born from 1960 to 1984. It is too early to distinguish whether there is a further improvement for patients born afterwards. The difference between the results reported from Italy and from Great Britain could be due to several factors, including different rates of compliance and a less uniform pattern of treatment. In fact, we evaluated data from seven large Italian centers, while the British study included patients belonging to different ethnic groups

and treated by more than a hundred different consultants. Data on adherence to treatment, unfortunately, are not available for our series of patients, but the importance of this aspect has been repeatedly demonstrated, even in a subgroup of our study population in whom survival at age 30 was 60% for compliant patients and 10% for the non-compliant ones.[14] The role of the different hemoglobin levels maintained by transfusion cannot be evaluated as it varied concomitantly in all centers, according to the recommendations of the national treatment protocols. Heart disease, including heart failure and arrhythmia, continues to be the leading cause of death, also for patients born after 1970, followed by infection. Of the 90 patients who were reported to have heart disease, 35 died of that complication, six died due to a different cause (1 diabetes, 1 septic shock, 1 unspecified inflammatory response, 1 anorexia, 2 thromboses) and 49 were still alive at the time of data collection. The role of aggressive therapy of myocardial disease cannot be established on the basis of the information available. However, intravenous or subcutaneous continuous chelation has been demonstrated to reverse established disease. The number of deaths due to infection should be decreasing as a result of immunization, prophylaxis and increased awareness of the risk. Eight deaths were due to malignancy. This number has not changed since our previous report,[5] making chance a likely explanation for what had been earlier considered a worrisome finding.

Despite the markedly improved survival, patients continue to be affected by a high number of complications. Heart failure and arrhythmia were significant factors predicting mortality.

HIV, which represented a tragedy of huge dimensions for hemophiliacs, did not have a great impact on the survival of thalassemia patients and should no longer represent a serious threat to multiply transfused patients.[15]

Thrombotic events were reported in 1.1% of the patients and caused 4.1% of the deaths. The increased frequency of this complication in both thalassemia major and intermedia has been recognized recently,[16] and the existence of prothrombotic hemostatic abnormalities has been demonstrated.[17]

More than half of the patients had delayed pubertal maturation. All of them, being born after 1970, started chelation therapy at the age of 10 or earlier, and no difference in frequency of pubertal maturation was found in the cohort born in 1970-75 with increasing year of birth. This is in contrast with data published by Bronspiegel *et al.*,[18] who found that 90% of the patients chelated before the age of 10 had normal pubertal development. Eleven percent of patients were affected by hypothyroidism. Conflicting percentages have been reported in the past[19] for this complication, which has been attributed to deposition of iron granules in the follicular epithelium, causing tissue fibrosis. The overall prevalence of diabetes, present in 6.4% of the patients, has also remained stable since the first detailed description in 1988.[20] However, when the number of patients developing diabetes, hypothyroidism, and hypogonadism were subdivided by birth cohort, a significant improvement was evident for the patients born more recently. In particular, the prevalence of diabetes was strikingly lower in patients born between 1975 and 1979 than it was in patients born in the years 1970-74. Surprisingly, diabetes was reported as the cause of death in 7 patients (3% of deaths). One patient developed diabetes at the age of 5.4, but it is improbable that iron overload played a role in this case. Liver disease was reported as the cause of death in 9 patients (4%), all born before 1970. The role of liver disease in this population of patients cannot be defined, because, at the time of the study design, this complication was not included among those to be reported by the centers. In fact, it was thought that, in the absence of histological data, the role of hepatitis could not be clearly defined. Infection with hepatotropic viruses is very common in Italian thalassemia patients. The Cooley Care Cooperative Group has reported the presence of hepatitis C virus (HCV) antibodies in 85% of multitransfused Italian patients[21] while a serologic pattern of previous hepatitis B virus (HBV) infection was found in 34%.[22] The striking improvement in survival for patients born after 1970 suggests an important role for iron chelation. In the past, survival has been shown to be related to the magnitude of body iron burden as determined quantitatively by hepatic iron concentration.[23] The protective effect of deferoxamine in preventing cardiac disease, glucose intolerance, diabetes and early death has also been detected in relatively small groups of patients.[12] The adequacy of serum ferritin to assess the iron burden has been disputed, because several variables can interfere with its reliability as a marker of iron overload. In fact, ferritin, being an acute phase reactant, is increased in chronic disease, malignancy, or inflammatory disorders, and in the presence of increased erythropoietin.[24] Ascorbic acid deficiency can lead to decreased synthesis and release of ferritin, even in the presence of massive iron stores.[25] Conversely, patients with active liver disease may have high serum ferritin levels that do not reflect the body iron load.[26]

In a retrospective study, Olivieri *et al.* demonstrated a better prognosis for survival without cardiac disease in transfused patients whose ferritin concentrations remained below 2,500 ng/mL.[27] In our population, a lower ferritin concentration predicted longer survival, and reduced risk of cardiac complications and of hypogonadism, both using the ferritin values directly, and when grouped to, below or above a threshold as low as 1,000

ng/mL. This finding can be of help, especially when direct measurements of hepatic iron are not feasible.

Females were found to have a better survival and a lower frequency of heart failure and arrhythmia. Although this could be due to better compliance on the part of females, such a hypothesis is not confirmed by ferritin levels, which are not significantly lower in females. A better life expectancy in females has also been observed in sickle cell anemia[28] and is the rule in the majority of the world populations.[29]

In conclusion, the results of this study show that mortality rates are decreasing in patients with thalassemia major, mainly as a consequence of better management of iron overload. Lower levels of ferritin are predictive of a better prognosis. Heart disease remains by far the most common cause of death and efforts should be directed towards the prevention of cardiac hemosiderosis. A reappraisal of the problem has recently been published.[30] Satisfactory compliance remains crucial for improving prognosis. Other causes of death, such as infection and thrombosis, may also decrease, as their importance is recognized. Complications are still frequent, but cardiac disease, hypogonadism, diabetes, and hypothyroidism are significantly less prevalent in the patients from cohorts born more recently. The information obtained can be of help in genetic counseling and when discussing the option of stem cell transplantation.

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