Survival and causes of death in 2,033 patients with non-transfusion-dependent β-thalassemia

Non-transfusion-dependent β-thalassemia (NTDT) is a broad term encompassing patients who do not require lifelong transfusion therapy for survival. NTDT patients commonly, but not exclusively, present to medical care later in childhood (commonly >2 years of age) and with milder anemia and clinical symptoms compared to patients with transfusion-dependent forms. Our understanding of the disease process in NTDT has evolved significantly over the past two decades and it is now established that a diagnosis of NTDT can be associated with greater morbidity than previously recognized.[1] Ineffective erythropoiesis and peripheral hemolysis lead to a state of chronic anemia, which can impact organ function in the long-term. There is a significant correlation between the degree of anemia and morbidity development in this NTDT patient population.[2] Ineffective erythropoiesis is also linked to other pathogeneses manifesting as extramedullary hematopoiesis, bone disease, hypercoagulability and vascular disease, as well as primary iron overload due to increased intestinal iron absorption.[1] Iron overload can be cumulative and leads to end-organ damage, especially in the liver.[3,4] Despite advances in realizing risk factors and morbidity in NTDT, data on mortality and causes of death remain limited.[5]

There are currently no approved drugs for the management of ineffective erythropoiesis, or anemia, in NTDT. Thus, despite the terminology, many patients are given sporadic transfusions or are even placed on regular transfusion programs later in their disease course. This is commonly undertaken in situations of acute stress (during pregnancy, surgery, or infection), in the context of supporting growth and development, or for the management and prevention of complications in adulthood.[6] Decisions are often based on the physician's judgement, since management guidelines have only become available since 2013 and do not necessarily provide specific recommendations for transfusion therapy or for other erythropoiesis modulators when data from clinical trials is absent.[7] Iron chelation therapy has been used in patients with NTDT for decades, but this have primarily based on expert opinion since data from dedicated clinical trials and management guidelines have only become available in the last 10 years.[8,9]

Against this background, the aim of the current study was to evaluate survival and causes of death in a large cohort of patients with NTDT. Considering the high variability in management practices, the impacts of transfusion and iron chelation therapy on mortality outcomes were also examined.

Data were retrieved from an International Health Repository (IHR) established and approved on 25 May 2017 by the Italian Ethical Committee (EudraCT and Sponsor's Protocol Code Numbers: 2017-004457-17 and 143AOR2017). All data were anonymized and added to the repository following informed consent by patients, or their legal representatives in case of death. The database included all β-thalassemia patients attending participating centers from 1 January 1997 onwards, and historic data were retrieved for all patients from birth up to 31 December 2020, in case of death, or loss to follow-up. The database included 13 international thalassemia centers of excellence from eight countries: Italy, Iran, Pakistan, USA, Oman, Egypt, Greece, and Saudi Arabia. For the current analysis, we gathered data on 2,033 patients identified by the centers as NTDT; a β-thalassemia diagnosis was confirmed by clinical and molecular studies at all participating centers. The definition of NTDT was based on the absence of dependence on transfusions for survival, delayed presentation, mild-moderate anemia, and clinician's judgement of disease severity at diagnosis and during follow-up. Patients had homozygous or compound heterozygous β-thalassemia mutations, or heterozygous β-thalassemia mutations combined with α-globin gene duplications (and hence an

Table 1. Causes of death in 2,033 patients with non-transfusion-dependent β-thalassemia.

Cause	n	% of deaths (n = 113)	% of population (n = 2,033)	Median age at death (min-max), years
Cardiovascular disease (iron-related cardiomyopathy, n = 2; other cardiomyopathy, n = 14; myocardial infarction, n = 1; valvular disease, n = 1; pulmonary hypertension, thrombosis or peripheral vascular disease, n = 23)	41	36.3	2.0	34.2 (19-85)
Hepatic disease (fibrosis or cirrhosis, n = 10; HCC, n = 13)	23	20.4	1.1	55.4 (26-76)
Cancer (solid or hematologic malignancy excluding HCC)	14	12.4	0.7	54.0 (12-85)
Infection	13	11.5	0.6	44.1 (12-68)
Unclassified thalassemia-related complications	17	15.0	0.8	19.8 (7-64)
Non-thalassemia related causes	5	4.4	0.2	62.0 (27-73)

HCC: hepatocellular carcinoma.

α/β-globin imbalance leading to clinically-significant disease). For each patient, data were retrieved for gender, age at last observation, status (living or dead) at last observation and eventual cause of death. Data was also gathered on whether the patient was transitioned to a regular transfusion program (and the date) or started receiving iron chelation therapy (and date).

Overall, 113 out of 2,033 patients (5.6%, 95.0% confidence interval [CI]: 4.6-6.6) died during the observation period. The median follow-up time was 33.9 years (interquartile range [IQR]: 23.7-46.8). The median age at death was 46.3 years (IQR: 28.3-61.9; 43.4% females), while the median age for patients alive at the last observation was 33.7 years (IQR: 23.7-45.9; 52.4% females). The Kaplan-Meier survival curve for all-cause mortality is illustrated in Figure 1A. Cumulative survival estimates at 18, 50, 65, 75, and 85 years were 99.4%, 93.4%, 81.8%, 66.2%, and 25.4%, respectively. By comparison, survival probability estimates at 50, 65, and 75 years in the normal population of Italy in 2019 were 98.5%, 94.0% and 82.9% (*http://dati.istat.it/*). Survival was significantly shorter in patients from the Middle East and Asia (n = 922) compared with the US and Europe (n = 1111), with a cumulative survival at 50 years of 74.9% vs 96.3%; Log-rank test χ^2: 82.581, p <0.001). Causes of death are summarized in Table 1. Cardiovascular disease was the leading cause of early death (36.3%, at a median age of 34.2 years), while hepatic disease was the leading cause of death in older patients (20.4%, at a median age of 55.4 years).

A subset of 254 patients (12.5%) were eventually placed on regular transfusion programs, starting at a median age of 10 years (IQR: 4-28.3). The remaining 1,779 patients (87.5%) received only sporadic or no transfusions at all. Survival was significantly worse in non-regularly transfused patients compared to regularly transfused patients for all-cause mortality (Log-rank test Chi-square: 13.298, *P*<0.001, Figure 1B). Cumulative survival estimates at 18, 50, 65, 75, and 85 years were 99.3% *vs.* 100%, 92.6% *vs.* 97.1%, 79.5% *vs.* 95.0%,

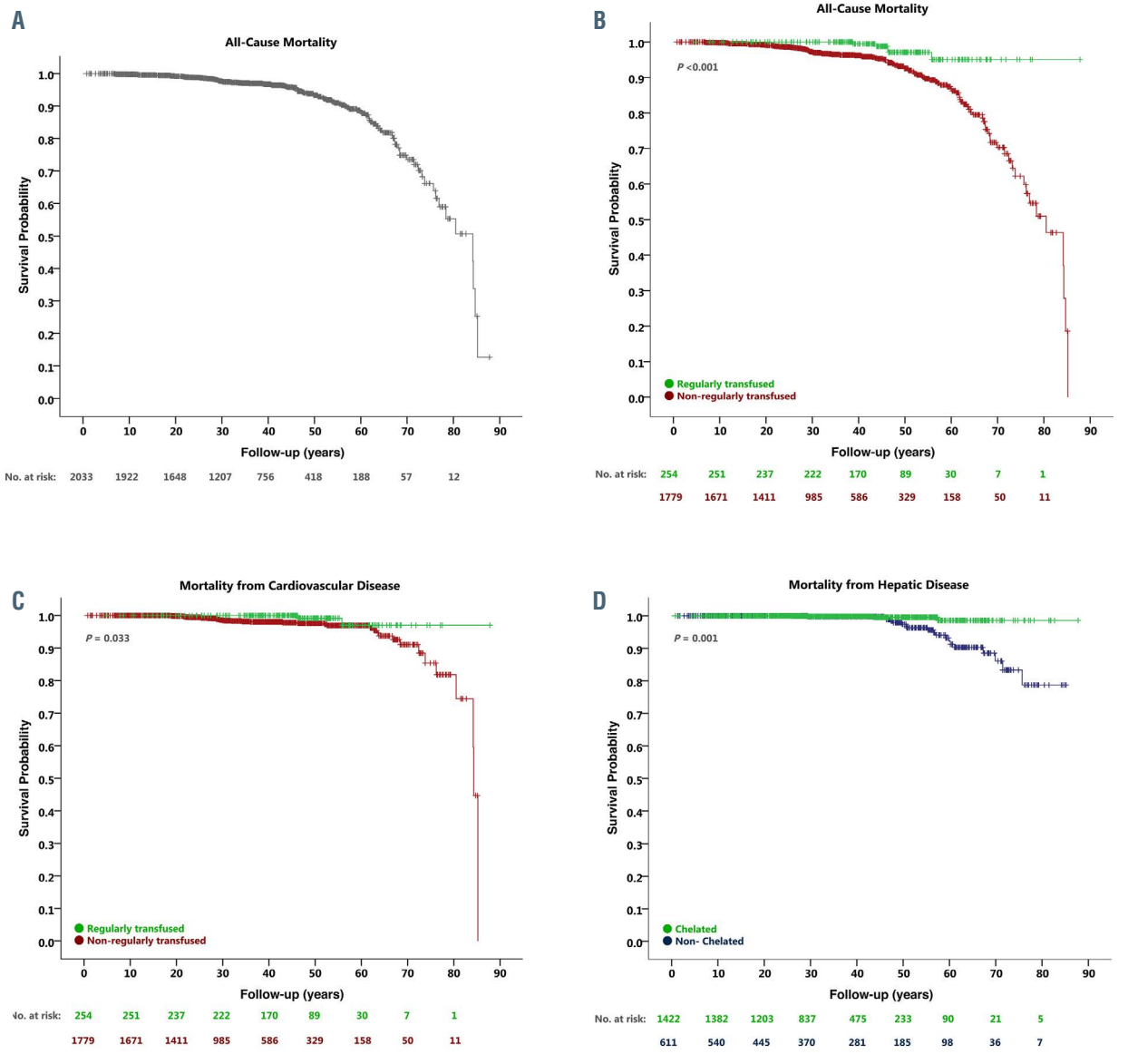

Figure 1. Kaplan-Meier survival curves. (A) all-cause mortality, (B) all-cause mortality according to regular transfusion therapy status, (C) mortality from cardiovascular disease according to regular transfusion therapy status, and (D) mortality from hepatic disease according to iron chelation therapy status.

62.2% *vs.* 95.0%, and 18.5% *vs.* 95.0%, respectively. Survival was also significantly worse in non-regularly transfused patients compared to regularly transfused patients when considering mortality from cardiovascular disease (Log-rank test χ^2: 4.571, *P*=0.033, Figure 1C) or hepatic disease (Log-rank test Chi-square: 4.989, *P*=0.026). Survival was comparable between splenectomized (n = 886) and non-splenectomized (n = 1,147) patients.

Iron chelation therapy was concomitantly used in all regularly-transfused patients and in 1,168 (67.7%) non-regularly-transfused patients, beginning at a median age of 15 years (IQR: 7-26). Survival was comparable in chelated and non-chelated patients for all-cause mortality (Log-rank test χ^2: 0.717, *P*=0.397). Survival was also comparable in chelated and non-chelated patients when considering mortality from cardiovascular disease (Log-rank test χ^2: 0.001, *P*=0.0969), but was significantly worse in non-chelated compared with chelated patients when considering mortality from hepatic disease (Log-rank test χ^2: 11.489, *P*=0.001, Figure 1D).

On multivariate Cox regression analysis, including regular transfusion and iron chelation as explanatory variables, regular transfusion therapy was associated with a reduction of approximately 80% in the risk of all-cause mortality (hazard ratio [HR]: 0.202, 95.0%CI: 0.080-0.509, *P*=0.001) and mortality from cardiovascular disease (HR: 0.199, 95.0%CI: 0.046-0.869, *P*=0.032); while iron chelation therapy was associated with a reduction of around 73% in the risk of mortality from hepatic disease (HR: 0.277, 95.0%CI: 0.093-0.830, *P*=0.022).

This is the first study to provide mortality estimates in a large cohort of NTDT patients. Cardiovascular disease was the leading cause of death, but unlike in patients with TDT, this cannot be fully explained by cardiac siderosis and subsequent heart failure secondary to chronic transfusions given that the cohort is not so transfusion-dependent. Chronic anemia and hypercoagulability can play a considerable role in the development of vascular disease in NTDT (large- and micro-vessel thrombosis, pulmonary hypertension, peripheral and renal vascular disease) with or without cardiac dysfunction. In fact, and as seen in regularly-transfused patients in this cohort, transfusions in this context may have a protective effect by halting ineffective erythropoiesis and subsequent pathogeneses; an observation made in previous cross-sectional studies.[6] Improvement in hemolysis markers, nucleated red cells and cardiac index have also been reported in longitudinal studies of NTDT patients who were started on regular transfusions in adulthood.[10] This also explains why iron chelation did not seem to have a role in preventing cardiovascular deaths in this cohort. Thus, a trial of chronic transfusion in patients at risk of significant morbidity may be justified but this needs to be weighed against the eventual risk of secondary siderosis and the elevated need and high cost of iron chelation therapy in a regular transfusion setting. We await data from various novel therapies targeting ineffective erythropoiesis and anemia in NTDT.[11]

Iron overload in non-regularly-transfused NTDT patients is attributed to hepcidin dysregulation and increased intestinal iron absorption.[12] Observational studies indicate that hepatic siderosis is the main consequence, with no evidence of iron deposition in the heart (unlike in transfusional siderosis).[1] Several reports have linked iron overload to hepatic fibrosis and hepatocellular carcinoma in NTDT;[13-15] these were a common cause of death in this cohort, although at older ages considering they require more time to manifest. Iron chelation was associated with a lower risk of death from hepatic disease, adding further evidence to data from clinical trials showing significant decline in liver iron concentration in NTDT patients receiving iron chelation.[8,9]

Our work merits further evaluation in prospective birth cohorts to address missing information and loss to follow-up bias typical of long-term retrospective studies, a factor that can lead to an over-estimation of survival risk. The study could also include additional subsets of non-transfusion-dependent patients including hemoglobin E/β-thalassemia and α-thalassemia, and further explore the role of genotype and environment in geographical variations in outcomes.

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