Morbidity and mortality of sickle cell disease patients is unaffected by splenectomy: evidence from three decades of follow-up in a high-income setting

Sickle cell disease (SCD) is a globally widespread hereditary red cell disorder characterized by the production of pathological hemoglobin S (HbS).[1] Patients with SCD include homozygous subjects for HbS (SS) and compound heterozygotes with HbS/HbC (SC) or HbS/$\beta^{+/0}$-thalassemia ($S\beta^0/\beta^+$). In Italy, SCD is endemic with HbS/$\beta^{+/0}$-thalassemia being prevalent in areas of southern Italy. In the last two decades, the number of SCD patients across Italy has increased due to migration from sub-Saharan Africa and the Middle East.[2,3] Italian expert centers for hemoglobinopathies, in which the vast majority of patients are managed, have registered about 2,300 patients with SCD, distributed over the whole territory with the highest prevalence in Sicily (10 patients/100,000 inhabitants) and in regions of the north of Italy (~5 patients/100,000 inhabitants) (*Online Supplementary Figure S1A*). In line with European Hematology Association guidelines, the main indications for splenectomy in SCD in Italy are splenic sequestration and hypersplenism.[4] Although studies on short-term post-splenectomy follow-up (e.g., 2-10 years) are available, results of long-term follow-on mortality are lacking.

Here we report on 11,195 patient-years of follow-up using a large cohort of SCD patients. We designed a retrospective observational cohort study, which was supported by the Italian Society of Thalassemia and Hemoglobinopathies (SITE; *www.site-italia.org*). We identified six reference centers of the Italian Hemoglobinopathy Comprehensive Care Network (*Online Supplementary Figure S1A*) with SCD patients followed from the 1990s with continuous follow-up data covering 30 years. The aim of the study was to compare survival, causes of death and complications in splenectomized *versus* not-splenectomized SCD patients. Data were collected between 2016-2018, curated and analyzed since then. Centers involved in the present study are geographically located in areas of high SCD prevalence and collectively follow up more than a third (n=801) (*Online Supplementary Figure S1B*) of registered SCD patients in Italy. Inclusion criteria were continuous long-term follow-up considered from the creation of the centers if the year of birth was before 1990 or from the first contact with the center before the age of 10 years.

For each patient, we collected data on gender, age at last follow-up, year of last follow-up, age of first access to the center, ethnicity, genotype ($S\beta^+$, $S\beta^0$ and SS, confirmed by molecular analysis), splenectomy, age and year of splenectomy, type of common therapy (chronic transfusion regimen, hydroxyurea or iron chelation treatment), age at first therapy, death, age and year of death, and cause of death. The definition of ethnicity was based on self-reported ancestry. No data on the method of splenectomy were available. The study was approved by the Ethics Committee of the Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy.

After exclusions, data from 534 patients (272 males, 51%) with genotypes $S\beta^+$ (n=171, 32%), $S\beta^0$ (n=176, 33%) and SS (n=187, 35%) were analyzed (Table 1, *Online Supplementary Figure S1B*). Gender was balanced overall and within the three genotypes considered. Altogether, in the period 1990-2018, 50 patients (10%) died, and 17 patients (3%) underwent their last visit before the start of survey in 2016 (lost to follow-up). The median follow-up was 26 years (interquartile range, 25th - 75th percentile, [IQR], 15-27 years; minimum – maximum, 1-28 years). Patients with the SS genotype - predominantly migrants from African countries and the Middle East - were younger than subjects with other genotypes (Table 1). A subset of 170 patients (32%), equally spread between males and females, underwent splenectomy. The age of splenectomy was similar between genders. The indications for splenectomy were acute splenic sequestration in 30/170 (17.6%), hypersplenism/recurrent splenic sequestration in 117/170 (68.8%), and unknown/other in 23/170 (13.5%). We found that SCD patients with the SS genotype were splenectomized earlier (7 years; IQR, 5-10 years; P<0.001) than those with either the $S\beta^0$ (11 years; IQR, 7.5-18.5 years; P=0.0024) or $S\beta^+$ genotype (20 years; IQR, 11-27 years). This is in line with previous reports in other cohorts of SS or $S\beta^0$ patients, for whom splenic sequestration is the main indication for splenectomy.[5-7] For the $S\beta^+$ genotype, the indication for splenectomy is hypersplenism more than splenic sequestration, which is consistent with the older ages observed. The probability of being splenectomized was greater in patients with $S\beta^0$ and $S\beta^+$ than in SS patients (P<0.001). Pairwise comparisons of proportions with the Bonferroni correction showed that the percentage of patients who underwent splenectomy was greater in the group with the $S\beta^0$ genotype (53%) than in the $S\beta^+$ group (34%; P=0.0012) and the SS group (9.6%; P<0.001) ($S\beta^0$ > $S\beta^+$ > SS) (Table 1). In our cohort, the rate of splenectomy in SS patients was close to that reported in other studies with a similar SS population (*Online Supplementary Figure S2*).[8-14] It is noteworthy that the rate of splenectomy in our

Table 1. Characteristics of the studied cohort of patients with sickle cell disease.

	Total	Sβ$^+$	Sβ^0	SS	*P*-value
Demographics					
N of patients (N of males)	534 (272)	171 (93)	176 (89)	187 (90)	0.7
Age in years at last follow-up, median (IQR)	38 (17-49)	39 (27-52)	42 (32-53)	18 (7-43)	<0.001
N of patients in age groups					
Age ≤19 years	151	32	19	100	<0.001
Age 20-39 years	138	55	50	33	0.005
Age 40-59 years	206	67	89	50	<0.001
Age ≥60 years	39	17	18	4	0.003
Follow-up in years, median (IQR)	26 (15-27)	26 (22-28)	26 (26-27)	17 (7-26)	<0.001
Splenectomy, N/total N in group	170/534	58/171	94/176	18/187	<0.001
Age at splenectomy in years, median (IQR)	13 (7-22)	20 (11-27)	11 (7.5-18.5)	7 (5-10)	<0.001
Ethnicity					
Caucasian, N	432	168	174	90	<0.001
African, N	99	3	2	94	<0.001
African-American, N	3	-	-	3	-
Deaths					
N of deaths	50	17	19	14	0.6
Splenectomy, N/N of deaths	20/50	6/17	11/19	2/14	0.04
Age at death in years, median (IQR)	49.5 (39-58)	49 (38-59)	48 (39-58)	50 (46-54)	0.9

IQR: interquartile range (25th - 75th percentile); ACS: acute chest syndrome.

Sβ patients was higher than that described by Belhani *et al.* and Diagne *et al.*, who carried out their studies on small Sβ patient populations in African countries.[9,12,14]

The long-term follow-up of our cohort of patients allowed us to analyze whether changes in the management of SCD (e.g. hydroxyurea or chronic transfusion regimen) affected the indication for splenectomy in SCD patients over time. To achieve this, we considered four different cohorts based on quartiles of the year of birth of patients (before 1966, 1967-1979, 1980-2000 and after 2001), each one including about 130 patients. The analysis (Figure 1A) suggested that indications for splenectomy did not change over time, being similar in different birth cohorts. Using the Kaplan-Meier method, the 10-year survival probabilities were estimated to be 87% (95% confidence interval [95% CI]: 81-93%), 86% (95% CI: 81-92), 83% (95% CI: 77-90%) and 88% (95% CI: 80-96%), respectively, for each of the four periods (*P*=0.71). This was confirmed when we analyzed the age-adjusted incidence rate of splenectomy over time considering different birth cohorts (Figure 1B). We then analyzed the survival rate and the causes of death within our SCD cohort. No statistically significant differences were observed in the survival or age of death between splenectomized and non-splenectomized patients with SCD (*P*=0.7 and *P*=0.9, respectively) (Figure 1C). The survival curves were similar for the three genotypes (*P*=0.29) with an overall median survival time of 72 years (Sβ^0: 73 years; Sβ$^+$: 68 years; SS: 68 years) (Figure 2A). As expected, the survival rate was significantly reduced in children splenectomized before 5 years of age, whereas no major differences were observed for the other age groups (Figure 2B). When we considered the impact of different treatments (chronic transfusion regimen, hydroxyurea or iron chelation treatment) *versus* no therapy on the mortality of patients with SCD, the mortality rate was worse in treated patients than in untreated ones (*Online Supplementary Figure S3A*). This might be related to the milder phenotype of untreated SCD patients compared to treated SCD subjects. Indeed, the percentage of sickle cell-related events was higher in treated SCD patients than in untreated ones (*Online Supplementary Figure S3B*). We registered 50 deaths, which occurred at a median age of 49.5 years (IQR, 39.1-57.5 years; minimum - maximum, 31-73 years), which was similar among genotypes (*P*=0.9). The four main causes of deaths were acute chest syndrome (n=15), liver failure (n=12), stroke (n=7) and solid cancer (n=7: 3 liver, 2 lung, 1 breast, 1 colon) (Figure 1D). Among the deaths due to liver failure, ten were related to chronic hepatitis C virus infection. In addition, two out of three patients with hepatocellular carcinoma had chronic hepatitis C virus infection. Splenectomy was reported in 20 out of 50 (40%) of the patients who died. Moreover, considering the subgroup of patients whose death was due to either acute chest syndrome, stroke or pulmonary

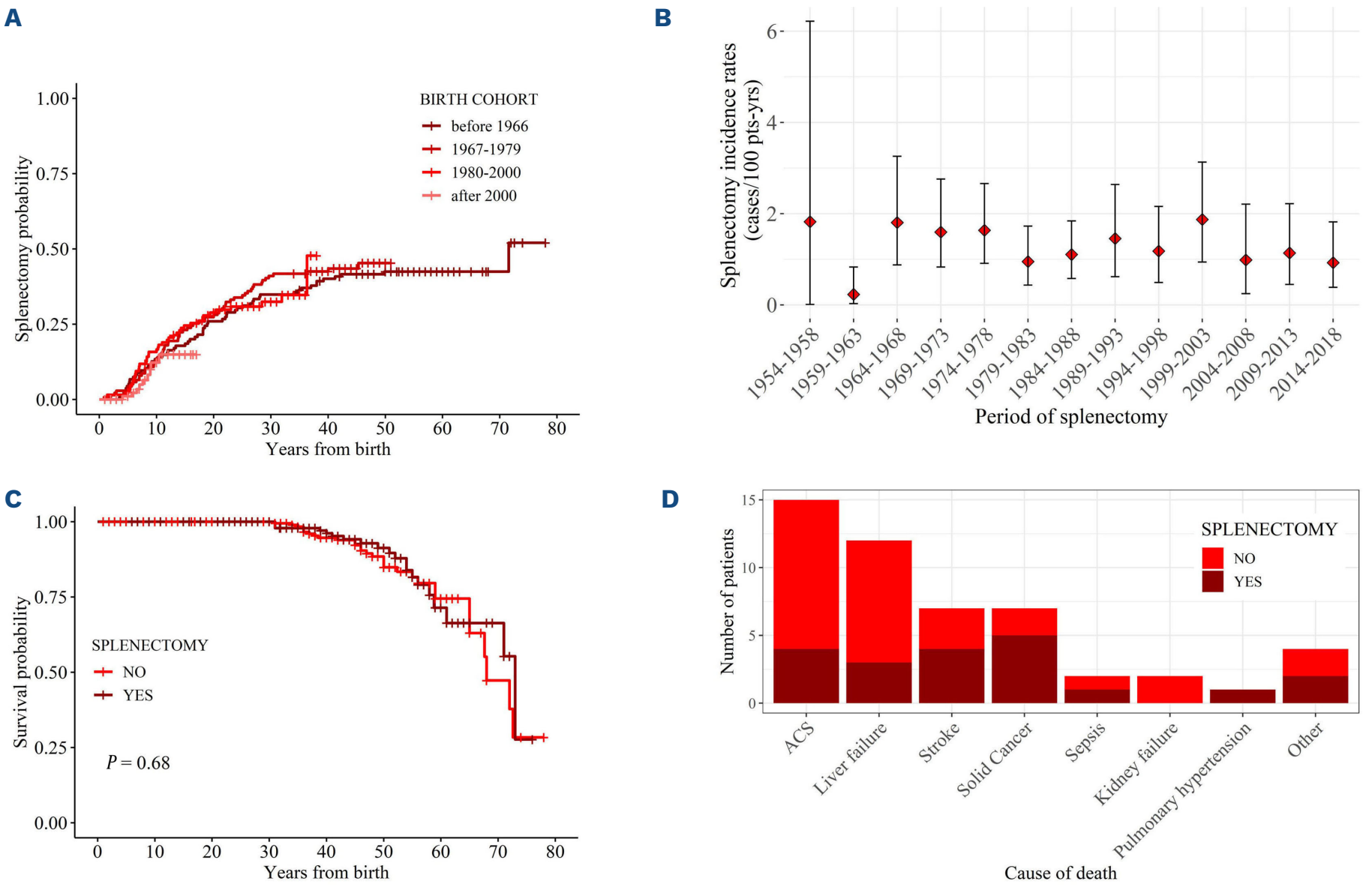

Figure 1. Splenectomy does not affect survival rate and the incidence of fatal infectious events in patients with sickle cell disease. (A) Probability of splenectomy in patients with sickle cell disease (SCD) analyzed for different birth cohorts (~130 patients/cohort). (B) Age-adjusted incidence rates of splenectomy in 5-year periods (mean value, 95% confidence interval). The age distribution of the SCD population in the period 2014-2018 was used to adjust rates. (C) Survival probability of patients with SCD according to splenectomy. (D) Causes of death in splenectomized (YES) or non-splenectomized (NO) patients with SCD. Pts-yrs: patient-years; ACS: acute chest syndrome.

hypertension, we did not observe a predominance of splenectomized patients *versus* non-splenectomized individuals (10 out 25, *P*=0.6). When we considered genotypes and causes of death in splenectomized SCD patients, we found that $S\beta^0$ patients had an increased risk of acute chest syndrome, liver failure and solid cancer compared to splenectomized SCD patients with either the SS or $S\beta^+$ genotype (*Online Supplementary Figure S3C*). Concerning the risk of death from sepsis, we did not find any difference between splenectomized and non-splenectomized patients with SCD. Our cohort of SCD patients received anti-pneumococcal, anti-meningococcal, anti-*Haemophilus influenzae* and anti-influenza virus vaccines. Based on our records, antibiotic prophylaxis was generally discontinued either after the age of 14 years or at 1 year after splenectomy, associated with education for the patients and caregivers. Our findings are concordant with results from four different studies which analyzed smaller SCD populations and for a shorter period of time compared to our study.[6,7,15,16] Similar results were also reported for two different studies from low-income countries with a follow-up of 18 months and 3 years after splenectomy.[10,14,17] In our cohort, the absence of a significant difference in fatal infectious events between splenectomized and non-splenectomized patients with SCD might be related to a combination of vaccination, patient education and the intensive follow-up program conducted in comprehensive care centers for hemoglobinopathies by expert medical staff. Although 3.4% of analyzed patients were born before 1980 and splenectomized before the age of 5 years, our results on fatal infectious events in splenectomized *versus* non-splenectomized patients were unaffected by including or excluding this population from our analysis. This was expected given that sickle cell patients are characterized by asplenia, which might expose them to an increased risk of infection compared to that of the healthy population. Overall, our data support the observation that patient education, vaccination programs and early identification and treatment of severe infections by expert medical staff help to prevent mortality due to sepsis.[18] The

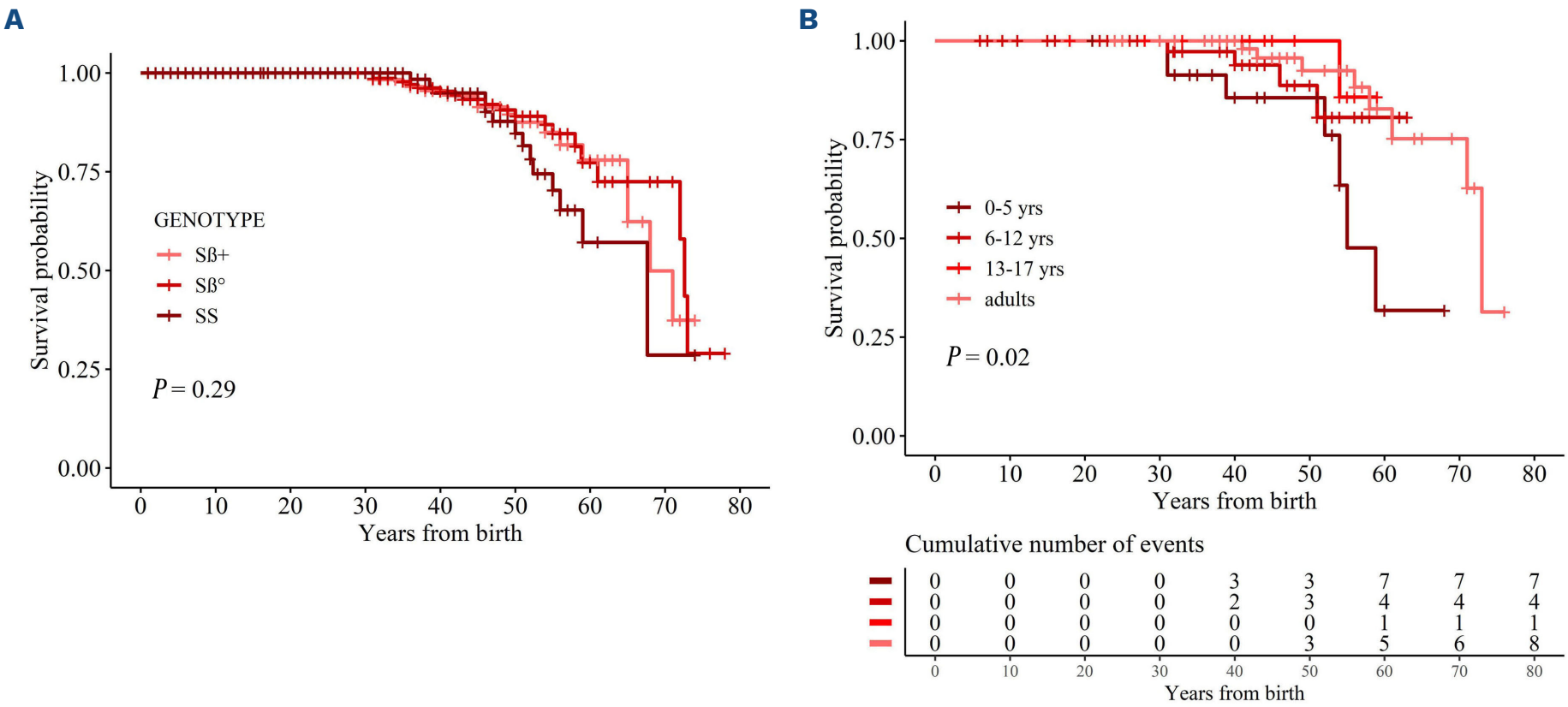

Figure 2. Probability of survival of patients with sickle cell disease. (A) Survival probability of patients according to genotypes. (B) Survival probability of patients with sickle cell disease who underwent splenectomy by age of splenectomy.

present study has some limitations due to its retrospective design (e.g., lack of details on surgical approaches and acute post-splenectomy complications) and a possible selection bias, our cohort being composed of well-treated patients followed from birth in comprehensive care centers for hemoglobinopathies.

In conclusion, this 26-year long-term follow-up cohort study of SCD patients highlights that Sβ patients require surgical splenectomy more frequently than SS patients, who in turn may undergo auto-splenectomy. The study provides crucial new evidence of the absence of negative impacts of splenectomy on fatal outcomes, supporting splenectomy as a recommended therapeutic approach in the treatment of patients with SCD.

Authors

Valeria Maria Pinto,[1*] Barbara Gianesin,[2*] Frédéric B. Piel,[3] Filomena Longo,[4] Paolo Rigano,[5] Alessandra Quota,[6] Vincenzo Spadola,[7] Giovanna Graziadei,[8] Filippo Mazzi,[9] Maria Domenica Cappellini,[8] Aurelio Maggio,[5] Antonio Piga,[10] Lucia De Franceschi[9#] and Gian Luca Forni[1#]

[1]Center for Microcythemia, Congenital Anemia and Iron Dysmetabolism, Galliera Hospital, Genoa, Italy; [2]ForAnemia Foundation, Genoa, Italy; [3]Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, UK; [4]Reference Center for Hemoglobinopathies, AOU San Luigi Gonzaga Hospital, Orbassano, Italy; [5]Campus of Hematology Franco and Piera Cutino, AOOR Villa Sofia-V. Cervello, Palermo, Italy; [6]Thalassemia Unit, P.O. Vittorio Emanuele III, Gela, Caltanissetta, Italy; [7]Thalassemia Center, P.O. Giovanni Paolo II, Ragusa, Italy; [8]Department of Medicine and Medical Specialities, IRCCS Ca' Granda Foundation, Maggiore Policlinico Hospital, Milan, Italy; [9]Department of Medicine, University of Verona & AOUI Verona, Policlinico GB Rossi, Verona, Italy and [10]Department of Clinical and Biological Sciences, University of Turin, Turin, Italy.

VMP and BG contributed equally as co-first authors.
#LDF and GLF contributed equally as co-senior authors.

Correspondence:
GIAN LUCA FORNI - gianluca.forni@galliera.it

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Contributions

VMP, LDF and GLF contributed to the conceptualization and design of the study; acquisition, curation, analysis and interpretation of the data; and writing, critically appraising, commenting on, reviewing and

editing the manuscript. FP and BG contributed to data analysis and interpretation; and writing, critically appraising, commenting on, reviewing and editing the manuscript. PR, AQ, CF, GG, FM, MDC, AU and AP contributed to the acquisition and curation of the data, and critical appraisal of and comments on the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data-sharing statement

Data are available on request to the authors.

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