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Morbidity and mortality of sickle cell disease patients is unaffected by splenectomy: evidence from 3 decades follow-up in a high-income setting

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Data will be available on demand

Authors Contributions
VMP, LDF and GLF contributed to the conceptualization and design of the study, acquisition and curation of the data, contributed to data analysis and interpretation, writing, critical appraisal and comments, reviewing and editing; FP and BG contributed to data analysis and interpretation, writing, critical appraisal and comments, reviewing and editing; PR, AQ, CF, GG, FM, MDC, AU, AP contributed to the acquisition and curation of the data, critical appraisal and comments. All authors have read and agreed to the published version of the manuscript.

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Running Title: Impact of splenectomy in SCD

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TO THE EDITOR,

Sickle cell disease (SCD) is a globally widespread hereditary red cell disorder characterized by the production of pathologic hemoglobin S (HbS)\(^1\). SCD includes homozygous subjects for HbS (SS) and compound heterozygotes HbS/HbC (SC) or HbS/\(\beta^+\)-thalassemia (S\(\beta^0/\beta^+\)). In Italy, SCD is endemic with HbS/\(\beta^+\)-thalassemia being prevalent in Southern Italian areas. In the last two decades, the number of SCD patients across Italy has increased due to migrations from sub-Saharan Africa and the Middle East\(^2,3\). Italian expert centers for hemoglobinopathies, where 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 the Northern regions (~5 patients/100,000 inhabitants) (Figure S1A). In line with EHA 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 (Figure S1A) with SCD patients followed from 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 vs 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 high-prevalence areas and altogether follow up more than a third (n = 801, 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 the last follow-up, year of the last follow-up, age of the first access to the center, ethnicity, genotype (\(\beta^+\), \(\beta^0\) and SS, confirmed by molecular analysis), splenectomy, age and year of splenectomy, type of common therapy (chronic transfusion regimen [CTR], hydroxyurea [HU] or iron chelation treatment [ICT]), age at first therapy, death, age and year of death, cause
of death. The definition of the 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 Sß+ (n=171, 32%), Sß0 (n=176, 33%) and SS (n=187, 35%) were analyzed (Table 1, 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 at follow-up). The median follow-up was 26 years (IQR: 15-27 years, min-max: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 also similar between genders. The indications for splenectomy were acute splenic sequestration 30/170 (17.6%), hypersplenism/ recurrent splenic sequestration 117/170 (68.8%), unknown/other 23/170 (13.5%). We found that SCD patients with the SS genotype were splenectomized earlier [7 years – IQR: 5-10 (p<0.001)] than either Sß0 [11 years – IQR: 7.5-18.5 (p=0.0024)] or Sß+ genotypes (20 years interquartile range (IQR): 11-27). This is in line with previous reports in other cohorts of SS or Sß0 patients, for which splenic sequestration is the main indication for splenectomy5–7. For the Sß+ genotype, the indication for splenectomy is hypersplenism more than splenic sequestration, which is consistent with the older ages observed. The probability to be splenectomized was greater in patients with Sß0 and Sß+ than in SS patients (p<0.001). Pairwise comparisons of proportions with Bonferroni correction show that the percentage of patients that underwent splenectomy was greater in patients with the Sß0 genotype (53%) than in the Sß+ group (34%, p=0.0012) and in the SS group (9.6%, p<0.001) (Sß0 > Sß+ > SS) (Table 1). In our cohort, the rate of splenectomy in SS patients was close to that reported in other studies with similar SS population (Figure S2)8–14. Noteworthy, the rate of splenectomy in our Sß patients was higher than that described by Belhani et al. and Diagne et al., who carried out their studies on smallest Sß patient population in African countries9,12,14.

The long-term follow-up of our cohort of patients allowed us to analyze whether changes in the management of SCD (e.g. HU or CTR) 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 indication for splenectomy did not change over time, being similar in different cohorts of birth. Using Kaplan-Meier method, the 10-year survival probabilities were estimated to 87% (95% CI: 81-93%), 86% (81-92), 83% (77-90%) and 88% (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 difference was observed in the survival or in the age of death between splenectomized vs 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 ($S^0$: 73 years; $S^+^*$: 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 difference was observed for the other age groups (Figure 2B). When we considered the impact of different treatments (CTR, HU or ICT) vs no therapy on the mortality rate of patients with SCD, the mortality rate was worse in treated than in untreated patients (Figure S3A). This might be related to the milder phenotype of untreated SCD patients compared to treated SCD subjects. Indeed, the percent of sickle cell related events was higher in treated SCD vs untreated SCD individuals (Figure S3B).

We registered 50 deaths with a median age at death of 49.5 years (IQR: 39.1-57.5 years; min-max: 31-73 years), similar among genotypes (p=0.9). The four main causes of deaths were ACS (n=15), liver failure (n=12), stroke (n=7) and solid cancer (n=7: 3 liver, 2 lung, 1 breast, 1 colon), respectively (Figure 1D). Among the deaths due to liver failure, ten were related to chronic-HCV-infection. In addition, 2 out of 3 patients with hepatocellular carcinoma had chronic-HCV-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 ACS, stroke or pulmonary hypertension, we did not observe a predominance of splenectomized patients vs non-splenectomized individuals (10 out 25, p=0.6). When we considered genotypes and causes of death in splenectomized SCD patients, we found that $S^0$ displayed increased risk of ACS, liver failure and solid cancer compared to splenectomized SCD patients with either SS or $S^+^*$ genotype (Figure S3C). Concerning the risk of death from sepsis, we did not find any difference between splenectomized and non-splenectomized patients with SCD. In our cohort, SCD patients received anti-pneumococcal, anti-
meningococcal, anti-

*Haemophilus influenzae* and anti-influenza virus vaccinations program. Based on our records, antibiotic prophylaxis was generally discontinued either after the age of 14 years or at one year after splenectomy, associated with patient and care-givers education. These agrees with results from four different studies, which analyzed smaller SCD populations and for a shorter period of time when compared to our study. Similar results were also reported in two different studies from low-income countries with a follow-up of 18 months and three years after splenectomy. In our cohort, the absence of significant difference in fatal infectious events in splenectomized vs non-splenectomized patients with SCD might be related to a combination of vaccination, patient education and the intensive follow-up program of comprehensive 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 vs non-splenectomized patients were unaffected by considering this population in or out of the analysis. This was expected given than sickle cell patients are characterized by asplenia, which might expose them to increased risk of infection compared to healthy population. Overall, our data support the observation that patient education, vaccination programmes and an early identification and treatment of severe infectious events by expert medical staff help prevent mortality due to sepsis. The present study shows some limitations due to the retrospective design (e.g. lacking of details on surgical approaches, acute post-splenectomy complications) and a possible selection bias, our cohort being composed of well treated patients followed from birth in comprehensive centers for haemoglobinopathies.

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


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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total</th>
<th>Sβ+</th>
<th>Sβ°</th>
<th>SS</th>
<th>p-value</th>
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<tr>
<td><strong>Demographic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Counts (males)</td>
<td>534 (272)</td>
<td>171 (93)</td>
<td>176 (89)</td>
<td>187 (90)</td>
<td>0.7</td>
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<tr>
<td>Median age (years) at last follow-up</td>
<td>38 (17-49)</td>
<td>39 (27-52)</td>
<td>42 (32-53)</td>
<td>18 (7-43)</td>
<td>&lt;0.001</td>
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<tr>
<td>Counts: age ≤ 19 yrs</td>
<td>151</td>
<td>32</td>
<td>19</td>
<td>100</td>
<td>&lt;0.001</td>
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<td>Counts: age 20-39 yrs</td>
<td>138</td>
<td>55</td>
<td>50</td>
<td>33</td>
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<tr>
<td>Counts: age 40-59 yrs</td>
<td>206</td>
<td>67</td>
<td>89</td>
<td>50</td>
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<tr>
<td>Counts: age &gt;60 yrs</td>
<td>39</td>
<td>17</td>
<td>18</td>
<td>4</td>
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<tr>
<td>Median years of follow-up (IQR)</td>
<td>26 (15-27)</td>
<td>26 (22-28)</td>
<td>26 (26-27)</td>
<td>17 (7-26)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Splenectomy (counts/total)</td>
<td>170/534</td>
<td>58/171</td>
<td>94/176</td>
<td>18/187</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age at splenectomy (years)</td>
<td>13 (7-22)</td>
<td>20 (11-27)</td>
<td>11 (7.5-18.5)</td>
<td>7 (5-10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
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<td>Caucasian</td>
<td>432</td>
<td>168</td>
<td>174</td>
<td>90</td>
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<td>African</td>
<td>99</td>
<td>3</td>
<td>2</td>
<td>94</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>African-American</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td><strong>Deaths</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of deaths</td>
<td>50</td>
<td>17</td>
<td>19</td>
<td>14</td>
<td>0.6</td>
</tr>
<tr>
<td>Splenectomy (counts/death)</td>
<td>20/50</td>
<td>6/17</td>
<td>11/19</td>
<td>2/14</td>
<td>0.04</td>
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<tr>
<td>Death age (years)</td>
<td>49.5 (39-58)</td>
<td>49 (38-59)</td>
<td>48 (39-58)</td>
<td>50 (46-54)</td>
<td>0.9</td>
</tr>
</tbody>
</table>

IQR: interquartile range, ACS: acute chest syndrome.
FIGURE LEGEND

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 analyzed for different birth cohort (~130 patients/ cohort).

(B) 5-years periods age adjusted incidence rates of splenectomy (mean value, 95% CI), the age distribution of SCD population in the period 2014-2018 was used as used to adjust rates.

(C) Survival probability of patients with sickle cell disease according to splenectomy

(D) Causes of death in splenectomized (YES) or not-splenectomized (NO) patients with sickle cell disease.

Figure 2. Survival probability of SCD patients.

(A) Survival probability of patients according to genotypes.

(B) Survival probability in SCD patients that underwent splenectomy by age of splenectomy.
Figure S1. A. Distribution of sickle cell disease (SCD) in Italy and comprehensive centers for hemoglobinopathies with long-term followed-up patients with SCD patients (n=2,300). B. Flow-chart of study population (SCD: Sickle Cell Disease).
**Figure S2.** Forest plot of observational studies reporting cases of surgical splenectomy in SCD for genotype SS (red) and Sβ, considered as Sβ° plus Sβ+ (gray). The studies reported were included in the systematic review of Ladu et al. (2021).
Figure S3. A. Survival probability for patients without therapy (NO) or treated with CTR, HU or ICT therapy. B. Percentage of sickle cell related events occurred in SCD patients by treatment type (HU/CTR/NONE). (CTR: chronic transfusion regimen; HU: hydroxyurea; ICT: iron chelation therapy). C. Causes of death in splenectomized (YES) or not-splenectomized (NO) patients with sickle cell disease according to genotypes.