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Long-Term Complications After Total Body Irradiation in Children Younger Than 4 Years Transplanted for Acute Lymphoblastic Leukemia

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Long-Term Complications After Total Body Irradiation in Children Youger than 4 years Transplanted for Acute Lymphoblastic Leukemia

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ABSTRACT

Total body irradiation (TBI)-based conditioning is the standard regimen for allogeneic hematopoietic stem cell transplantation (HSCT) in high-risk or relapsed pediatric acute lymphoblastic leukemia (ALL) but is variably used before age 4 because of concerns about long-term toxicity. Long-term physical, educational, and quality-of-life outcomes were assessed in patients from the French long-term follow-up program LEA who underwent TBI-based HSCT for ALL at 2 to <4 years of age (younger group, n=37) and compared with those of matched prepubertal older children transplanted at ≥4 years (older group, n=105). After a mean follow-up of 15.6 years, the number of long-term complications was similar in the two groups (3.3 *vs* 3.2, p=0.90). Among the 20 complications analyzed, pulmonary dysfunction was the only event that differed significantly, being more frequent in the older group (43% *vs* 19%, p=0.01). In a multivariate multistate model, age group was not associated with a higher risk of transition to a greater complication burden (HR 1.05, 95% CI 0.83-1.32; p=0.70). Educational outcomes and quality of life were comparable between groups. These findings do not suggest greater long-term toxicity in children undergoing TBI-based HSCT before 4 years of age and support reconsideration of the lower age limit for its use.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is indicated for treating pediatric high-risk or relapsed acute lymphoblastic leukemia (ALL). Two types of myeloablative conditioning are used before allogeneic HSCT: total body irradiation (TBI)-based regimens and chemotherapy alone. Survivors of childhood leukemia who reach adulthood are at risk of long-term chronic health conditions due to the disease and therapy.[1] In patients undergoing HSCT, the conditioning regimen is a major determinant of long-term complications.[2] TBI is associated with an increased risk of late effects, particularly in young children, including infertility, growth retardation, and subsequent malignant neoplasms (SMNs).

The FORUM study, a landmark multinational randomized study, compared TBI-based conditioning with chemotherapy-only conditioning in pediatric ALL patients >4 years old. This trial showed that TBI plus etoposide significantly improved 2-year overall survival (OS, 91%) compared with chemotherapy-only conditioning (75%), while also reducing the risk of relapse.[3] In this trial, children <4 years were excluded from TBI-based conditioning because of concerns about excessive toxicity and late complications. In this subgroup, two TBI-free chemotherapy regimens were compared prior to HSCT: fludarabine (Flu) and thiotepa (Thio) combined with either busulfan (Bu) or treosulfan (Treo). Outcomes were notably less favorable than in older children receiving TBI, with a 3-year OS of 63% for Flu/Thio/Bu and 76% for Flu/Thio/Treo. The cumulative incidence of nonrelapse mortality (NRM) was low (6% of patients receiving Flu/Thio/Bu and 3% of those receiving Flu/Thio/Treo), but relapse was the major cause of treatment failure, with a cumulative incidence at 3 years of 42% and 45%, respectively.[4] These data underscore the need to reconsider the optimal age threshold at which the benefit–risk balance of TBI becomes acceptable.

Long-term complications after HSCT in very young children have been described in retrospective studies including heterogeneous cohorts with different underlying diseases and conditioning regimens, with or without TBI.[5-7] These studies helped establish TBI as a major risk factor for long-term complications. However, they were not specifically designed to evaluate TBI, but rather the overall impact of HSCT in young children. In addition, the effect of age on long-term complications was assessed indirectly, with age generally included as a covariate in multivariate models rather than being the focus of a dedicated analysis. In the present study, we report the first large homogeneous cohort of children with ALL receiving TBI-based conditioning with prospective follow-up and specifically compare long-term toxicity between children aged 2 to <4 years and those aged ≥4 years.

PATIENTS AND METHODS

LEA project

The LEA program (*clinicaltrials.gov* identifier: NCT01756599) is a national French cohort initiated in 2004 to assess long-term health status, quality of life (QoL) and socioeconomic status of patients treated during childhood or adolescence for acute leukemia after 1980 in 19 cancer centers in France. Follow-up begins 1 year after HSCT. Clinical examinations are conducted at predefined intervals: every 2 years until the age of 20 and every 4 years thereafter. Long-term complications are detected by physicians through medical examinations and adequate additional tests (biological and radiological) as previously described.[8,9]

Patients

Patients were eligible if they were included in the LEA program up to March 2025 and had undergone HSCT with a TBI-based conditioning regimen for ALL. Patients were

classified into two age groups: the younger group included those aged 2 to <4 years at the time of HSCT, while the older group comprised prepubertal patients aged 4 to <9 years for girls and 4 to <10 years for boys. The study focused on patients aged <10 years to limit potential bias related to differences in first-line treatment strategies in older children. Patients were excluded if they had Down syndrome, received ≥ 2 HSCT, or had CNS-directed irradiation administered as part of therapy preceding HSCT. For each younger patient, up to three children from the older age group were selected as matched controls based on same sex, similar history of relapse, and comparable time from HSCT to the last evaluation.

Clinical data related to HSCT were obtained from the LEA database, including patient demographics, complete remission (CR) status (including any relapse), details of HSCT procedures (*i.e.*, conditioning regimen, donor type, source of stem cells, and graft-versus-host disease (GvHD) grading, if any) and long-term complications. The study was approved by the French National Program for Clinical Research, the French National Cancer Institute (InCA), and the review boards of the institutions involved. All patients provided written informed consent for participation in the study.

Outcomes

The prevalence of twenty complications, defined in Supplementary Table 1, were analyzed : alopecia, bone defects (osteonecrosis, osteoporosis), cardiomyopathy, cardiovascular risk factors or complications (coronary artery disease, diabetes, metabolic syndrome, obesity, stroke), chronic kidney disease, endocrine complications (gonadal dysfunction and hypothyroidism), sensory defects (cataract, hearing loss), iron overload, pulmonary dysfunction, neurological complications, SMNs and meningioma, short stature and underweight.

Socioeconomic characteristics and educational outcomes were self-reported by participants' parents, or by the participants themselves when they were aged 18 years or older. Socioeconomic characteristics included parental educational level and household financial situation. Educational outcomes included grade retention, implementation of academic support, and changes in the patient's educational track (shift from general to vocational education). The highest educational level attained by the patient was assessed at the last follow-up.

The QoL was assessed using the SF36 questionnaire in patients who had reached adulthood.[10,11]

Statistical analysis

Descriptive statistical analyses were performed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). Qualitative data were expressed as counts and percentages. Quantitative data were presented as mean and standard error of mean (SEM). Fisher's exact tests were used to compare qualitative variables, while the Student's *t*-test or the Mann-Whitney U test was employed for quantitative variables. A multistate model was used to analyze the accumulation of long-term complications over time, using time since diagnosis as the timescale. Analyses were performed in R v4.5.2 using the mstate package (v0.3.3) to construct the multistate data structure. Six successive health states were defined: no complication, one complication, two complications, three complications, four complications, and five or more complications. A time-to-event model was fitted to estimate cause-specific hazard ratios (HRs) according to covariates, while unobserved transitions were treated as right-censored. A semi-parametric proportional hazards model was used, with a common baseline hazard across all transitions. Transition-specific effects were incorporated through the covariate structure. The effect of age group on the transitions was assessed after

adjustment for sex, age at HSCT, history of relapse, and chronic graft-versus-host disease. A p-value < 0.05 was considered statistically significant.

RESULTS

Study cohort

Among 705 patients aged 2 to <10 years for boys and 2 to <9 years for girls who underwent HSCT for ALL in the LEA cohort up to March 2025, 588 (83%) received TBI-based conditioning. Of these, 35 were excluded according to pre-specified criteria (Table 1). The remaining eligible patients were stratified by age at HSCT into a younger group (2 to <4 years, n=37) and an older group (≥4 years, n=516). After matching, 37 younger patients were compared with 105 older patients; 31 were matched to three controls each and 6 to two controls.

Data on socioeconomic characteristics and educational outcomes, derived from patient-reported questionnaires, were available for 127/142 patients (younger group: 33/37, 89%; older group: 94/105, 90%). Among these, 74 were still attending school at the time of the last evaluation (younger group: 25/33, 76% *vs* older group: 49/94, 52%), while 53 had completed their schooling (8/33, 24% *vs* 45/94, 48%, p = 0.02). Analysis of educational outcomes was restricted to the latter subgroup, *i.e.*, patients who were no longer in education.

Patient and HSCT characteristics

Baseline patient and leukemia features, along with key HSCT-related characteristics, are detailed in Table 1. The mean age at HSCT was 3.2 years in the younger group and 7.2 years in the older group. The proportion of male patients was similar between the two groups (59% of younger *vs* 63% of older children, p = 0.84). The mean duration from HSCT to last follow-up was 15.6 ±0.7 years (16.9 ±1.6 years in the younger group

and 15.2 ±0.8 years in the older group, p = 0.29). At the time of HSCT, 43% of younger patients and 46% of older patients had a history of leukemic relapse (p = 0.85). Cumulative anthracycline exposure, expressed in doxorubicin-equivalent doses,[12] tended to be higher in younger children than in older children (251 ±24 mg/m² *vs* 208 ±11 mg/m²), but the difference was not statistically significant (p = 0.07).

Conditioning regimens were similar between groups (p = 0.87), with TBI + cyclophosphamide and TBI + etoposide being the most frequently used in both younger (38% and 30%) and older children (45% and 24%). TBI-based preparative regimens reflected the protocols in use at the time of transplantation. Overall, all patients received fractionated TBI, and the vast majority (138/142, 97%) were treated with the commonly used 12 Gy schedule, consisting of a total dose of 12 Gy delivered in 6 fractions (*i.e.* 2 Gy twice daily for 3 days) with lung shielding at 8 Gy. In the younger group, 33 of 37 patients received this regimen, whereas 4 received fractionated schedules with total doses of 11 Gy (n=1) or 10 Gy (n=3). In the older group, all 105 patients received the 12 Gy fractionated schedule.

Stem cell source distribution was comparable between the two groups, with genoidentical sibling donors being the most frequent, accounting for 51% in younger and 40% in older children (p = 0.56).

Acute GvHD (grade ≥2) affected 51% and 50% of patients (p = 0.85), while chronic GvHD occurred in 11% and 18%, respectively (p = 0.30).

Incidence of long-term complications

The number of long-term follow-up assessments was similar between groups, with approximately one-third of patients undergoing ≥ 5 evaluations (p = 0.65). Among the 20 long-term complications studied, the number of complications per patient at last follow-up ranged from 0 to 9 and did not differ between groups, with a mean of 3.3 in

younger patients and 3.2 in older patients (Supplementary Figure 1, Table 2; p = 0.9). The proportion of patients with ≥5 complications was likewise similar (35% in younger *vs* 30% in older patients).

The most frequent complications were (percentages are presented as younger *vs* older patients): short stature (72% *vs* 63%), cataracts (70% *vs* 58%), hypothyroidism (46% *vs* 34%), and gonadal dysfunction. In females, gonadal dysfunction was more frequent in older than in younger patients (50% *vs* 77%; p = 0.08), whereas in males an opposite trend was observed (59% *vs* 40%; p = 0.16). Overall, there were no significant differences in the prevalence of complications between the two groups except for pulmonary dysfunction, which was more frequent in the older group (19% vs 43%, p = 0.01) (Table 2).

Subsequent malignant neoplasms were reported in 14 patients (6/37 in the younger group [16%] and 8/105 in the older group [8%], p = 0.2). Tumors observed were, in the younger group: thyroid tumor (n=4) and basal cell carcinoma (n=2), and in the older group: meningioma (n=3), thyroid tumor (n=2), oral cavity squamous cell carcinoma (n=2) and pleural mesothelioma (n=1). The median interval between HSCT and tumor occurrence was 13.7 years (range 7.1 - 33.2 years).

Global accumulation of long-term complications

The accumulation of the 20 complications was assessed in younger and in older children using a multivariate multistate model (Figure 2). Using this model, belonging to the younger *vs* older group was not significantly associated with the probability of transition to a higher state of complications, after adjustment for sex, age at HSCT, history of relapse, and chronic graft-versus-host disease (HR 1.05; 95% CI 0.83-1.32; p = 0.70).

Because a small number of patients in the younger group received TBI doses other than 12 Gy, a sensitivity analysis restricted to patients treated with the standard 12 Gy fractionated regimen was performed for both the long-term complication incidence analysis and the multistate model. This analysis yielded findings similar to those of the main analysis (data not shown), including for secondary tumors: 4/33 (12%) in the younger group *vs* 8/105 (8%) in the older group (p=0.48).

Educational trajectory

In the subgroup analysis restricted to patients who were no longer in education at the time of the last evaluation (Table 3), no statistically significant difference was found between groups for parental educational level (p = 0.66) or parental financial situation (p = 1). In the younger and older groups, respectively, parental educational level was high school diploma or lower in 50% and 63% of cases, and higher education in 50% and 38%. Families were described as financially comfortable in 80% and 77% of cases, whereas financial difficulties were reported in 20% and 23%. Regarding the highest educational level attained, 43% of patients in the younger group and 59% of those in the older group had reached higher education, whereas 57% and 42%, respectively, had attained a high school diploma or lower. This difference was not statistically significant (p = 0.68).

Health-related quality of life

Supplementary Table 2 summarizes health-related QoL outcomes self-reported by 69 patients who reached adulthood after undergoing HSCT at 2 to <4 years of age (n = 14) or at ≥ 4 years of age (n = 53). The QoL outcomes were adjusted for potential confounding factors, specifically age at evaluation and sex. Overall, although no differences reached statistical significance across any of the analyzed domains, patients included in the younger group tended to have slightly higher scores in social

and psychological domains, including social life (β = +9.2) and mental health (β = +9.1), compared with older patients. In contrast, scores for physical-related domains, including physical abilities and vitality, were similar between the two age groups. In addition, patients in the younger group reported slightly better outcomes for physical pain (β = +7.9).

DISCUSSION

Improved survival in pediatric ALL has shifted attention toward long-term treatment-related complications,[1] which now represents a major clinical challenge. Survivors of childhood HSCT often experience long-term complications in multiple organ systems.[13,14] Very young patients are thought to have increased vulnerability to treatment-related toxicity, and as a result, most protocols restricted the use of TBI-based conditioning to patients older than 4 years. This study provides insights into the frequency of long-term complications in children aged 2-4 years by comparing them with those older than 4 years. Overall, this study shows that the burden of long-term complications in children receiving TBI-based conditioning was comparable between the two age groups, suggesting that, despite this presumed increased risk, long-term toxicity is not substantially greater in this population. Our results complement the companion study from Eichinger *et al.*, published in the same issue of the journal, which compared TBI- and chemotherapy-based conditioning in children aged 2–4 years and found better disease control and survival with TBI, at the cost of more subsequent malignant neoplasms. Together, these data help refine the debated benefit-risk balance of TBI in this age group.

Among all the long-term complications analyzed, pulmonary dysfunction was the only one that was significantly more frequent in one group, occurring more often in older

than in younger children (43% *vs* 19%, p = 0.01). This age-dependent effect has been previously reported in pediatric cohorts of long-term HSCT survivors. Inaba *et al*. evaluated pulmonary function in 89 children after HSCT for hematologic malignancies and showed that pulmonary dysfunction was strongly associated with older age at HSCT, with patients >12.7 years at transplantation demonstrating no evidence of functional recovery on long-term follow-up. In contrast, younger children may exhibit partial recovery over time, possibly reflecting age- and hormone-dependent mechanisms of lung growth and repair.[15] In a larger cohort of 311 patients aged 0.6–25 years, Liu *et al*. confirmed that age is an independent risk factor for long-term pulmonary dysfunction in multivariate analyses, and additionally reported no difference in rates of pulmonary dysfunction between TBI- and busulfan-based conditioning regimens.[16] This suggests that long-term complications are not uniquely attributable to irradiation exposure but rather result from multifactorial post-transplant processes, as well as cumulative pre- and post-transplant exposures to other treatments.

TBI-based conditioning regimens have been shown to significantly impair growth in prepubertal patients through combined damage to the hypothalamic–pituitary axis, as well as to gonadal and thyroid function and the epiphyseal growth plates of the spine.[17] Short stature is the most frequent long-term complication, observed more often in children aged 2 to <4 years than in those aged ≥4 years, although this difference was not statistically significant (72% *vs* 63%, p = 0.4). This concerning complication warrants regular endocrinological follow-up in all children. Growth hormone deficiency is common and should be actively screened for, as it allows consideration of growth hormone therapy, which may limit the negative impact on adult height.[18,19]

Gonadal dysfunction is frequently observed after myeloablative conditioning and may lead to impaired pubertal development, sexual dysfunction, and infertility. Age at HSCT

and sex are key determinants of gonadal toxicity.[20] We previously demonstrated a continuous effect of age at HSCT on the prevalence of gonadal dysfunction in female patients, with significantly higher rates of ovarian function preservation in those treated before the age of 5, regardless of conditioning regimen (TBI-based or chemotherapy-only).[21] Consistently, in the present cohort, gonadal dysfunction was more frequent in older *vs* younger girls (77% *vs* 50%, p = 0.09), although this difference did not reach statistical significance, likely due to the small number of younger patients included. In male patients, pubertal status at the time of HSCT appeared to be the most important determinant of gonadal outcome.[22] Prepubertal boys were at higher risk of long-term gonadal impairment, with increased rates of testosterone replacement therapy in adulthood and a higher risk of infertility.[23-25] All patients in this cohort were prepubertal at the time of HSCT. However, we observed a nonsignificant trend toward higher rates of gonadal dysfunction in the younger compared with the older patients (59% *vs* 40%, p = 0.16), although no such intra-prepubertal age gradient has been clearly demonstrated in previous reports. This finding may warrant further investigations.

A relatively high rate of SMNs was observed in our cohort compared with previous studies, reaching 10%. This finding may be explained by two factors. First, our definition of SMNs included meningiomas. Although histologically benign, these tumors may lead to significant long-term morbidity and were therefore considered clinically relevant. Second, SMNs may occur very late after HSCT. Previous studies showed that the incidence of most SMNs does not plateau over time.[26] Consistently, the relatively long follow-up in our cohort (mean 15.6 years) likely contributed to the higher observed prevalence, in line with the long latency to tumor development in this population (median 13.7 years). In the largest study evaluating the risk of SMNs in a cohort of 3291 childhood ALL survivors, Westerveld et al. reported 10 SMNs among the 160

children undergoing HSCT with TBI-based conditioning without prior cranial radiotherapy, corresponding to an incidence of 6.3%. The authors observed no difference in SMN occurrence according to conditioning regimen, including TBI-based *vs* non-TBI approaches. In addition, age at diagnosis (and by extension age at transplantation) was not identified as an independent risk factor for SMNs.[27] The spectrum of SMNs observed in our cohort was consistent with tumors previously reported after TBI exposure, including thyroid malignancies, head and neck carcinomas, and basal cell carcinomas.[28] Thyroid cancers appeared relatively frequent in the younger group, in line with previous studies identifying younger age at exposure as a risk factor for radiation-associated thyroid malignancies.[29, 30] Although the higher incidence of SMNs in the younger group (16% *vs* 8% in the older group) did not reach statistical significance, it nonetheless highlights the importance of rigorous long-term surveillance, particularly for thyroid tumors, which appeared to be overrepresented in this group.

Among patients who had completed their education, no significant differences were observed between younger and older children for grade retention, academic support, change in educational track, or educational attainment. This is broadly consistent with the literature, which indicates that pediatric HSCT survivors may develop cognitive and school difficulties overall, although these impairments are generally limited and not consistently driven by age alone. Phipps et al. reported a less favorable trajectory in younger children, whereas Willard et al. showed that the impact of age was more nuanced and interacted with TBI exposure; similarly, Perkins et al. found largely average global intellectual functioning even in children transplanted before 3 years of age.[31-33] Data from the present study do not support a specific detrimental effect of

TBI-based HSCT performed between 2 and 4 years of age on educational outcomes, although the small number of evaluable younger patients warrants caution.

Overall, the present study confirmed the toxicity of TBI-based conditioning but did not show greater long-term toxicity in children transplanted before 4 years of age. These findings support reconsideration of the lower age limit for its use, given its established benefit in reducing relapse and improving survival, and may help inform the design of future HSCT trials in the pediatric population.

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Authorship contributions: QN performed research, analyzed data and wrote the paper; PS, GM, JHD, JB designed and supervised the research, and revised the manuscript; PA analyzed data and revised the manuscript; CD, CPo, SV, CR, AB, MF, VG, MP, CJ, SD, CPa, LOG, AT, CAA, JK, STD, MVB, CT, MDT, GL collected data and revised the manuscript. All authors participated sufficiently in the work to take responsibility for appropriate portions of the content and gave final approval of the manuscript to be published.

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FIGURE LEGENDS :

Figure 1: Flowchart of patient selection and matching

Flowchart of patient selection from the L.E.A. cohort of children with acute lymphoblastic leukemia who underwent HSCT with a TBI-based conditioning regimen up to 2025. Patients with Down syndrome, ≥ 2 HSCT, or prior CNS irradiation were excluded. Eligible patients were grouped by age at HSCT: 2 to <4 years (younger group) *vs* 4 to <9 years (girls), 4 to <10 years (boys) at HSCT (older group). Patients 2-4 years old were matched to up to three older children based on sex, relapse history, and time from HSCT to last evaluation.

ALL: acute lymphoblastic leukemia, CNS: central nervous system, HSCT: hematopoietic stem cell transplantation, LEA: french « *Leucémies de l'Enfant et de l'Adolescent »* program, TBI: total body irradiation, y: years.

Figure 2: Probability of transition to a higher number of long-term complications

The curves represent the estimated probability of transitioning to an additional long-term complication (from no complication to more than five long-term complications), as a function of time since diagnosis (years) in the younger group (2 to <4 years at HSCT; left) and in the older group (4 to <9 years for girls, 4 to <10 years for boys at HSCT; right). Each line between two areas represents the probability of developing an additional long-term complication over time since leukemia diagnosis.

Figures

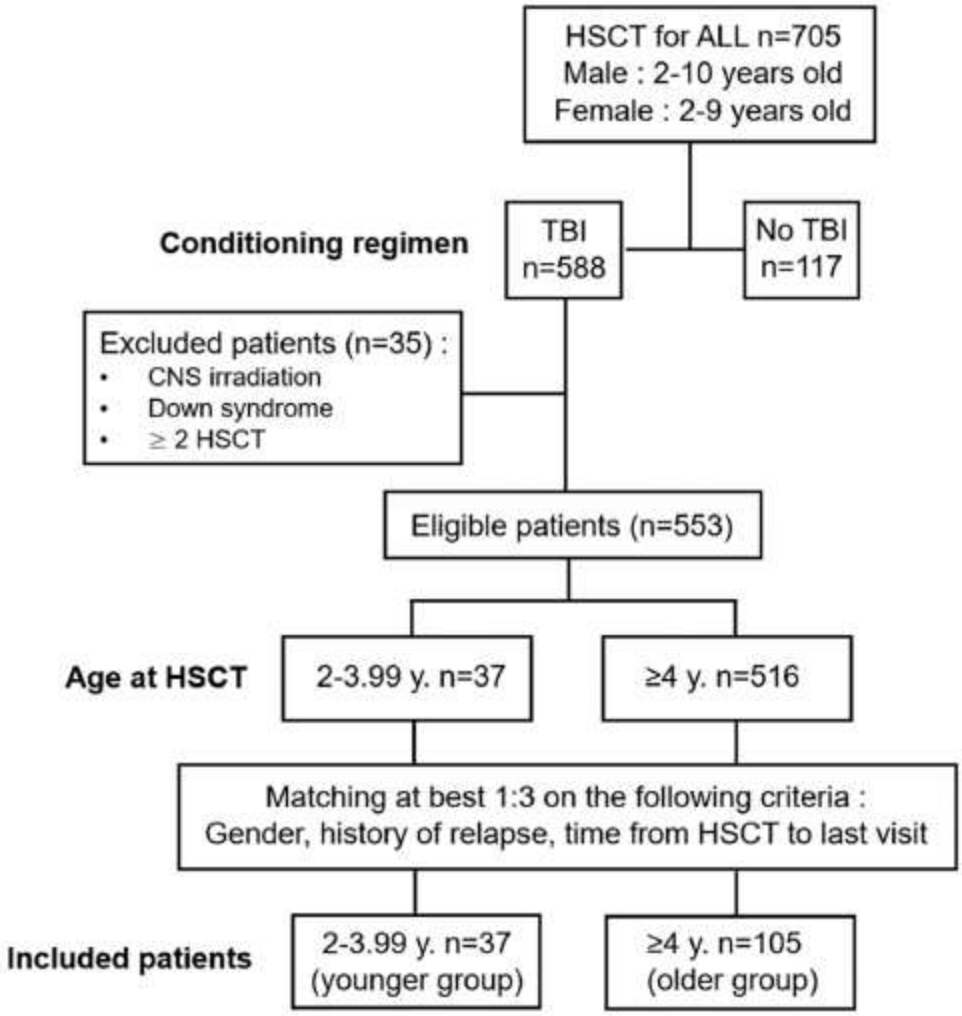


Figure 1

Flowchart of patient selection and matching

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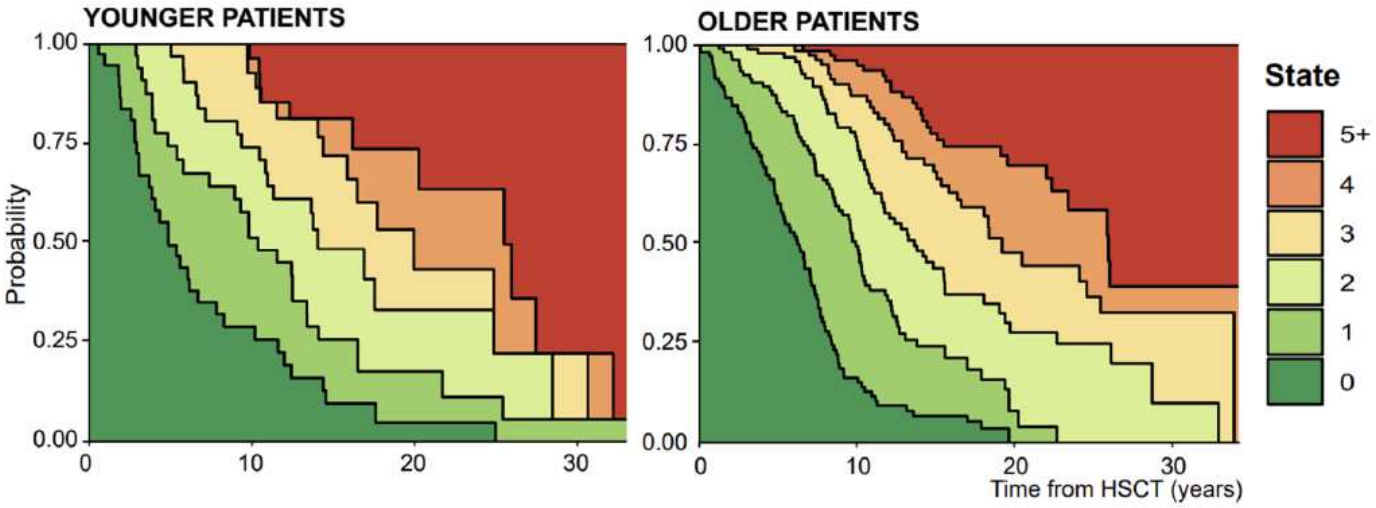


Figure 2

Probability of transition to a higher number of long-term complications

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