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Acute leukaemia

Transplant-related toxicity and mortality: an AIEOP prospective study in 636 pediatric patients transplanted for acute leukemia

A Balduzzi[1], MG Valsecchi[13], D Silvestri[1], F Locatelli[2], L Manfredini[3], A Busca[4], AP Iori[5], C Messina[6], A Prete[7], M Andolina[8], F Porta[9], C Favre[10], S Ceppi[11], G Giorgiani[2], E Lanino[3], A Rovelli[1], F Fagioli[4], C De Fusco[12], R Rondelli[7], C Uderzo[1] for the Associazione Italiana Ematologia Oncologia Pediatrica-BMT Group

[1]*Clinica Pediatrica, Università degli Studi di Milano-Bicocca, Centro Trapianto Midollo Osseo, Monza, Italy;* [2]*Dipartimento di Scienze Pediatriche, Università di Pavia, Unità di Oncoematologia e Trapianto di Midollo Osseo, Policlinico S Matteo, Pavia, Italy;* [3]*Istituto G Gaslini, Unità Trapianto Midollo Osseo, Genova, Italy;* [4]*Dipartimento di Scienze Pediatriche e dell'Adolescenza, Università degli Studi di Torino, Torino, Italy;* [5]*Cattedra di Ematologia, Università degli Studi di Roma, Roma, Italy;* [6]*Clinica di Oncoematologia Pediatrica, Università di Padova, Padova, Italy;* [7]*Clinica Pediatrica, Policlinico S Orsola-Malpighi, Università degli Studi di Bologna, Bologna, Italy;* [8]*Clinica Pediatrica Centro Trapianti, Istituto per l'Infanzia Burlo Garofolo, Trieste, Italy;* [9]*Clinica Pediatrica, Università di Brescia, Spedali Civili di Brescia, Brescia, Italy;* [10]*Clinica Pediatrica, Università di Pisa, Pisa, Italy;* [11]*Unità Organica di Pediatrica, Reparto di Onco-Ematologia – TMO, Ospedale R Silvestrini, Perugia, Italy;* [12]*Dipartimento di Ematologia Pediatrica, Ospedale Pausilipon, Napoli, Italy; and* [13]*Dipartimento di Medicina Clinica e Biotecnologia Sanitaria, Università degli Studie di Milano-Bicocca, Milano, Italy*

Summary:

Hematopoietic stem cell transplantation can cure high-risk acute leukemia (AL), but the occurrence of non-leukemic death is still high. The AIEOP conducted a prospective study in order to assess incidence and relationships of early toxicity and transplant-related mortality (TRM) in a pediatric population. Between 1990 and 1997 toxicities reported in eight organs (central nervous system, heart, lungs, liver, gut, kidneys, bladder, mucosa) were classified into three grades (mild, moderate, severe) and prospectively registered for 636 consecutive children who underwent autologous (216) or allogeneic (420) transplantation, either from an HLA compatible related (294), or alternative (126) donor in 13 AIEOP transplant centers. Overall, 47% of the patients are alive in CR (3-year EFS: 45.2%, s.e.: 2.1), 19% died in CR at a median of 60 days (90-day TRM: 14.3%, s.e.: 1.4), 34% relapsed. Toxicity of any organ, but mucosa and gut, was positively correlated with early death; moderate and severe toxicity to heart, lungs, liver and kidneys significantly increased early TRM, with estimated relative risks of 9.1, 5.5, 2.7 and 2.8, respectively, as compared to absent or mild toxicity. Patients with grade III–IV aGVHD experienced more than double (56% *vs* 19%) TRM than patients with grade 0–II aGVHD. A higher cumulative toxicity score, estimating the impact of toxicity on TRM, was significantly associated with transplantation from an alternative donor. Quantitative assessment allowed us to describe the extent to which 'grade' of toxicity and 'type' of involved organs were related to mortality and pre-transplant characteristics and yielded a prognostic score potentially useful to compare different conditioning regimens and predict probability of early death.
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Correspondence: Dr A Balduzzi, Clinica Pediatrica dell'Università di Milano – Bicocca, Ospedale S Gerardo di Monza, Via Donizetti, 106 – 20052 Monza (Milano) Italy
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Hematopoietic stem cell transplantation is currently used to treat childhood acute leukemia (AL) when chemotherapy yields a poor outcome.[1–11] Chemotherapy cures more than 70% of children with acute lymphoblastic leukemia (ALL), while relapses, particularly those occurring early after diagnosis and involving the bone marrow, may benefit from bone marrow transplantation. Transplantation is commonly adopted as a front-line strategy for very high risk ALL and for acute non-lymphoblastic leukemia (ANLL), since it is expected to improve the outcome, as compared to conventional chemotherapy. A compatible related donor, usually an HLA identical sibling, would be the best option; the outcome after autologous transplantation and transplantation from alternative donors is still jeopardized by a high risk of relapse in the former and of transplant-related toxicity (TRT) in the latter. Although relapse is the most frequent cause of transplant failure, the antileukemic effect of conditioning regimens, administered to eradicate leukemia and suppress host hematopoiesis, is not likely to be further increased without increasing toxicity, and possibly jeopardizing the final outcome, since many patients still die of TRT, despite supportive therapy and improvements in

infectious disease management in the last decade.[1,12–30] Since appropriate tools for measuring toxicity would allow comparison of different conditioning regimens, Bearman and collaborators[12] retrospectively registered toxicity reported in eight organs in a series of 195 leukemic patients, transplanted in Seattle up to 1988. The four-grade classification proposed by the Seattle team was restricted to conditioning regimen side-effects and excluded events related to infections, acute graft-versus-host disease (aGVHD) and drugs used for supportive therapy.[12] Each patient's 'cumulative toxicity', defined by the sum of the maximum grade of toxicity experienced in each organ, did not account for the role of each organ and duration toxicity. Clinicians know that severe and long-lasting toxicities, involving more than one crucial organ, such as lungs and liver, are expected to be lethal more often than mild and brief toxicities to the bladder or mucosae, but the extent to which 'grade' and 'duration' of toxicity, as well as 'number' and 'type' of involved organs are related to mortality, has not yet been described by quantitative methods.

We report the results of the prospective study 'Early Toxicity after Transplantation', conducted by the AIEOP (Associazione Italiana di Ematologia ed Oncologia Pediatrica) in order to (1) assess early toxicity and transplant-related mortality (TRM) in a pediatric population transplanted for AL in recent years; (2) study the relationship between early toxicity reported in the first 3 months in each organ and TRM; (3) develop a system to assess 'cumulative toxicity' as a risk factor for TRM and study the relationship between pre-transplant characteristics and cumulative toxicity.

Patients and methods

Patients

Toxicities after transplantation were prospectively registered with homogeneous criteria from January 1990 in the AIEOP transplant centers, which enroll more than 90% of the Italian pediatric population transplanted for malignancies. Six hundred and forty-two consecutive patients transplanted for AL were registered up to December 1997 by 14 centers; since one center, reporting six patients, did not specify toxicity data, this report includes the 636 evaluable patients (62% male; median age at the time of transplant: 8 years, range: 0.4–18) transplanted in 13 AIEOP centers, with a median follow-up of 3.5 years. Patients transplanted for ALL were 410 (64%) (277 in 1st or 2nd complete remission (CR); 133 with active or advanced disease (AD)), and for ANLL 226 (36%) (162 in 1st CR; 64 with AD). The source of hematopoietic stem cells was bone marrow in 588 patients, peripheral blood in 32 and cord blood in 16. Two hundred and sixteen patients (34%) received autologous transplantation, and 420 (66%) were given an allograft. Donors were HLA compatible relatives for 294 and alternative (52 partially mismatched related, 74 volunteer unrelated) donors for 126 children. Conditioning regimens included chemotherapy only in 196 (31%) patients; total body irradiation (TBI), usually delivered over 3 days twice daily for a total of 1200 rad, was given with one or more cytotoxic drugs in 129 (20%) and 311 (49%) patients, respectively. Chemotherapy only containing regimens included busulfan (16 mg/kg over 4 days, adjusted by levels in about half of the patients) and cyclophosphamide (120 mg/kg over 2 days) (25%), either alone (11%) or in combination with melphalan (140 mg/m^2) (8%) or vepeside (30 mg/kg) (6%). The single drugs associated with TBI were melphalan (140 mg/m^2), vepeside (40 mg/kg), cyclophosphamide (120 mg/kg over 2 days) or cytarabine (100 mg/kg), overall in 20% of the patients; the third drug most commonly used in association with TBI and cyclophosphamide was thiotepa (20%), vincristine (15%), or vepeside (6%). Half of the autologous grafts were purged, mostly by ASTA-Z. In the allogeneic setting GVHD prophylaxis mostly consisted of cyclosporine only (1–3 mg/kg i.v.) in patients transplanted from an HLA compatible related donor, and of cyclosporine (2–3 mg/kg i.v) plus 'short-methotrexate' in patients transplanted from alternative donors; for some of them T cell depletion was adopted *in vitro* (10%) or *in vivo* (33%), mostly using Campath and antilymphocytic serum, respectively.

Toxicity assessment

Myeloid engraftment was defined as occurring on the first of 3 consecutive days with ANC $>0.5 \times 10^9$/l. AGVHD was classified according to the Seattle criteria.[1,30] Early toxicities of eight organs (central nervous system (CNS), heart, lungs, liver, gut, kidneys, bladder, mucosa) were classified in three grades (I: mild, II: moderate, III: severe) according to the classification shown in Table 1, modified after Bearman for a pediatric population.[12] Severe toxicities which led to death *per se* were marked as 'lethal', and, similarly to Bearman's grade four toxicity, they were still included in grade three for the purpose of the analysis. Whenever it was possible to differentiate, events due to infections or aGVHD were not included. Toxicities reported from the beginning of the conditioning regimen up to 90 days after transplantation were considered in this study. Dates of toxicity onset and resolution and maximum grades for each organ were to be reported by the clinicians in charge on appropriate forms, which were centralized yearly at the AIEOP Registry. Dates of onset or resolution of toxicity were missing in one-third of the cases; as no reliable retrospective completion of these variables was possible, we were not able to take into account toxicity duration in this analysis. Inconsistent, unlikely, outlier and missing data were checked with each center, to ensure data quality.

Statistical analysis

Descriptive statistics were used to depict reported toxicity per organ and grade. The cumulative incidence of TRM was estimated accounting for the time from transplantation to non-leukemic death, adjusting for relapse as a competing event.[31] The Kaplan–Meier estimate of event-free survival (EFS) was calculated by considering the time from transplant to relapse or death, whichever occurred first.[32] Time was censored at the time of last follow-up if none of the events considered had occurred. Follow-up was updated as of March 1998 and the minimum potential follow-up was

Table 1 Toxicity grading[a]

CNS	1. easily woken somnolence, mild EEG changes, transient diplopia; 2. heavy somnolence, confusional status, neurological impairment, optical neuritis; 3. seizures, coma.
Heart	1. mild EKG or X-ray changes, arrythmias, transient hypertension; 2. same abnormalities as above but more severe and requiring treatment; 3. unresponsive cardiac failure.
Lungs	1. asymptomatic X-ray changes; 2. dyspnea and hypoxia (percutaneous O_2 saturation less than 90%) due to disventilation; 3. ARDS, need of mechanical ventilation.
Liver	1. bilirubin 2–5 mg/dl, AST/ALT 80–200 U/l, liver enlargement <1 in, weight gain <10%; 2. bilirubin >5 mg/dl, AST/ALT >200 U/l, liver enlargement >1 in, weight gain >10%, ascites; 3. severe VOD, liver failure, coma
Gut	1. vomiting, diarrhea less than 30 ml/kg, abdominal pain; 2. hematemesis, melena, diarrhea more than 30 ml/kg and/or persisting >10 days; 3. ileus.
Kidneys	1. increased creatinine, less than twice basal value; 2. increased creatinine, more than twice basal value; 3. dialysis.
Bladder	1. macro-hematuria; 2. hemorrhagic cystitis; 3. severe hemorrhagic cystitis (>10 days).
Mucosae	1. mild stomatitis; 2. severe stomatitis requiring morphine (15 days); 3. intubation.

[a]Organ impairments due to infection or GVHD were not included in the transplant-related toxicity for the purpose of this analysis.

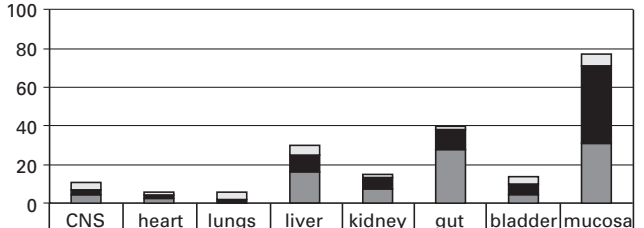
3 months after transplantation. The probability of failure was defined as the quantity '1-EFS'. A logistic model was used to evaluate the relationship between degree of toxicity reported for each organ and the occurrence of non-leukemic death within 90 days after transplantation.[33] A general model was fitted including one binary indicator for each organ, with code 'zero' (reference category) for absent or mild (grade one) toxicity, and code 'one' for moderate or severe toxicity (grades two and three). The estimates of the odds ratio approximated the relative risk of early non-leukemic death of patients having moderate or severe toxicity in one organ *vs* those having absent or mild toxicity in the same organ. Since the effects of toxicities occurring in more than one organ were likely to be more than additive, first order interactions between covariates were analyzed; since none emerged as significantly associated with the odds of death, they were not included in the final model. A more complex model, with two dummy variables for each organ, with the same reference category, but with separate codings for grades two and three was also fitted to the data, to look for trends according to grades. A score expressing the estimated cumulative impact of toxicity on early TRM was calculated as the value of the linear predictor: the score for each individual was a weighted sum of the toxicity grade for each organ, with weights equal to the regression coefficients estimated by the first model.[33] A higher score accounted for a higher risk of early non-leukemic death. The question of whether a higher score might be explained by the patient's pre-transplant characteristics was addressed by fitting a linear model to the score itself, as a function of the following covariates, each one listed with the proper number of dummy variables: age (0: <3 years, 1: 3–10 years, 2: >10 years), diagnosis and phase risk category (0: ALL in 1st or 2nd CR and ANLL in 1st CR; 1: ALL in AD, ANLL in 2nd CR or in AD), type of transplant and donor (0: autologous, 1: allogeneic from related compatible donor, 2: allogeneic from alternative donor), type of conditioning regimen (0: chemotherapy only, 1: TBI plus one drug, 2: TBI plus two or more drugs). The purpose of the linear model was to test whether pre-transplant characteristics were predictive of cumulative toxicity.

Results

Toxicity and TRM

Ninety-six percent of the patients reached myeloid engraftment at a median of 16 (range: 3–70) days after infusion; the 24 patients who did not engraft died at a median of 22 days after infusion, mostly (63%) of infection. Forty-eight percent of the allogeneic transplant patients developed grade II–IV aGVHD at a median of 11 days. Incidence of grade II–IV aGVHD was similar for patients transplanted from HLA compatible related or alternative donors; incidence of severe aGVHD (graded III–IV) was 14% and 24%, respectively ($P = 0.01$). Organ toxicities per grade are shown in Figure 1. Most frequent toxicities were reported in mucosae or gut, respectively in 77% and 40% of the patients; however, severe toxicity in these organs was limited to 6% and 2% of the patients. Twenty-nine percent of the patients experienced some toxicity in one organ only, 24% in two organs, 15% in three organs, and 16% in four or more organs; no toxicities were reported in 16% of the patients. Thirty percent of grade three toxicities were reported as lethal, mainly in the CNS, heart, lungs and liver.

The outcome of this series of patients, according to diagnosis and type of transplant, is reported in Table 2. Overall, 47% of the patients (301/636) are alive and in continuous CR and the 3-year EFS was 45.2% (s.e.: 2.1). Nineteen percent (119/636) of the patients died in remission and median time to death was 59 days (range: 2–1587) after



	CNS	heart	lungs	liver	kidney	gut	bladder	mucosa
□ III	3.5	1.4	3.9	4.6	1.6	1.6	3.1	6.4
■ II	2.0	1.6	1.6	8.8	6.0	10.4	6.4	39.2
▒ I	5.5	2.8	0.6	16.5	7.4	27.7	4.1	31

Figure 1 Incidence of toxicity by grade for each organ: histogram and table with percent frequencies.

Table 2 Transplantation outcome: incidence of events according to diagnosis and phase, and type of transplant

Type of transplant and donor	*Total (% of the total)*	*Dead in CR (%)*	*Relapsed (%)*	*Alive in CR (%)*
Overall	636 (100)	119 (19)	216 (34)	301 (47)
ALL	410 (64)	88 (21)	144 (35)	178 (44)
Autologous				
I–II CR	72 (11)	2 (3)	39 (54)	31 (43)
AD	21 (4)	2 (10)	12 (57)	7 (33)
Total	93 (15)	4 (4)	51 (55)	38 (41)
Comp. related				
I–II CR	152 (24)	18 (12)	42 (28)	92 (60)
AD	60 (9)	16 (27)	27 (45)	17 (28)
Total	212 (33)	34 (16)	69 (33)	109 (51)
Alternative				
I–II CR	53 (8)	22 (41)	12 (23)	19 (36)
AD	52 (8)	28 (54)	12 (23)	12 (23)
Total	105 (16)	50 (48)	24 (23)	31 (29)
ANLL	226 (36)	31 (14)	72 (32)	123 (54)
Autologous				
I CR	90 (14)	3 (3)	32 (36)	55 (61)
AD	33 (5)	6 (18)	16 (49)	11 (33)
Total	123 (19)	9 (7)	48 (39)	66 (54)
Comp. related				
I CR	62 (10)	7 (11)	9 (15)	46 (74)
AD	20 (3)	4 (20)	9 (45)	7 (35)
Total	82 (13)	11 (13)	18 (22)	53 (65)
Alternative				
I CR	10 (2)	4 (40)	2 (20)	4 (40)
AD	11 (2)	7 (64)	4 (36)	0 —
Total	21 (4)	11 (52)	6 (29)	4 (19)

ALL = acute lymphoblastic leukemia, ANLL = acute non-lymphoblastic leukemia; CR = complete remission; AD = advanced disease (ie other than I or II CR for ALL and other than I CR for ANLL); Comp. related = compatible related donor; alternative = unrelated donor or related partially mismatched donor.

transplantation, with 90 events occurring within 90 days, and four after 1 year. Infections were the most common cause of death (41%), with a quarter of the cases due to fungi. Toxicity and hemorrhages accounted for the remaining 40% and 16% of the causes of death, which were not reported in 3%. A grade three toxicity was reported as lethal in 8% of the patients, all but one being lethal within the first 90 days; among causes of death, half of the lethal toxicities were reported in the lungs and were described as ARDS or interstitial pneumonia. Seventy percent of the 101 patients who developed toxicity in four or more organs died in remission. TRM was 14.3%, (s.e.: 1.4) at 90 days and 18.9% (s.e.: 1.9) at 3 years overall; TRM at 90 days and at 3 years according to type of transplant and donor is shown in Figure 2. TRM was associated with type of transplant; a Cox model pointed out that the hazard of non-leukemic death for conventional allogeneic and alternative donor transplant was 2.6 and 11.4 times higher than for autologous transplants. Patients with grade III–IV aGVHD experienced more than double (56% *vs* 19%) transplant-related deaths than did patients with grades 0–II aGVHD. Relapse after transplantation occurred in 216 (34%) patients, in particular in 46% of the autotransplant patients, and in 28% of the allotransplant patients. Table 3 reports

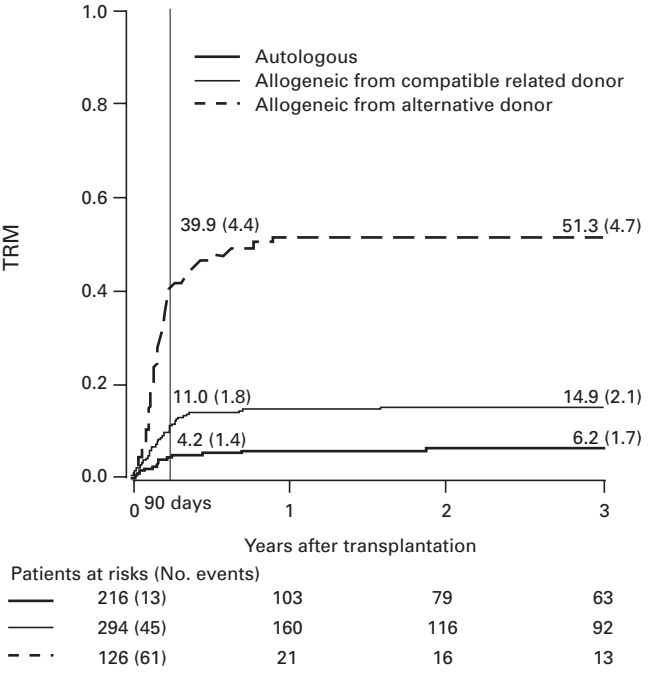


Figure 2 Kaplan–Meier estimated transplant-related mortality according to type of transplant and donor. The values reported on the curves (standard error) refer to 90 days and 3 years after transplantation.

Table 3 Estimates and standard error (s.e.) of TRM at 90 days and probability of failure from any cause at 3 years according to diagnosis and type of transplant

	TRM at 90 days (s.e.)	*Probability of failure[a] at 3 years (s.e.)*
Overall	14.3 (1.4)	54.8 (2.1)
Diagnosis, phase		
ALL	17.3 (1.9)	60.1 (2.6)
in I,II CR	11.8 (2.0)	51.6 (3.2)
advanced disease	28.9 (4.0)	77.8 (3.9)
ANLL	8.9 (1.9)	46.0 (3.5)
in I CR	5.0 (1.7)	34.6 (3.9)
advanced disease	18.8 (4.9)	71.0 (5.9)
Type of transplant		
Autologous	4.2 (1.4)	53.8 (3.5)
Allogeneic	19.5 (1.9)	55.3 (2.6)
Related compatible donor	11.0 (1.8)	46.2 (3.1)
Alternative donor	39.9 (4.4)	78.0 (4.1)

[a]Probability of failure = 1 – EFS.

the TRM at 90 days and the probability of failure from any cause, either relapse or death, at 3 years. Forty-seven (11%) patients underwent a second transplant, 86% for relapse; 12 (26%) of them survive.

Relationship between early organ toxicity and TRM

The relative risks of early death related to the presence of moderate or severe toxicity *vs* absent or mild toxicity in each organ, estimated by the logistic model, are reported

in Table 4. Toxicity of any organ, except for mucosae and gut, was positively correlated with early death. Moderate and severe toxicity of heart, lungs, liver and kidneys significantly ($P < 0.05$) increased early TRM, with estimated relative risks of 9.1, 5.5, 2.7 and 2.8, respectively, as compared to absent or mild toxicity. The logistic model discriminating between moderate and severe toxicity (data not shown), highlighted, as expected, a higher impact on TRM of the higher grades. In particular, severe lung toxicity was related to a 11-fold increase in the risk of death ($P < 0.001$), and severe CNS toxicity was related to a three-fold increase in the risk of death ($P = 0.02$), compared to absent or mild toxicity. When the subgroup of the allogeneic transplant patients was analyzed, results were consistent with the general model shown in Table 4, with relative risks slightly higher for lung toxicities, and slightly lower for heart and kidneys. To evaluate a possible influence of misclassification of liver toxicity instead of aGVHD, the subgroup of allogeneic transplant patients who did not develop grades II–IV overall aGVHD was considered ($n = 219$); the relative risks estimated for all organs were consistent with those of the general model. The distribution of liver and lung toxicities, either moderate or severe, was similar in this subgroup of patients compared to the overall sample, despite the association with TRM, and did not reach statistical significance.

In terms of homogeneity issues, distributions of toxicities per organ and grade were roughly similar among centers; what is more important, the association between toxicity and TRM was homogeneous among centers, as the parameter estimates for each organ did not change their value or significance when the logistic model was adjusted according to center, and no center turned out to be significantly different from the others.

Relationship between pre-transplant characteristics and cumulative toxicity

The cumulative impact of toxicity on TRM was represented by the individual score obtained as the weighted sum of the toxicity codes, weighted by the estimated coefficients shown in Table 4. The mean cumulative toxicity score was 0.25 (s.d.: 0.98) in the overall sample; a higher cumulative toxicity score was related to a higher risk of early non-leukemic death. In particular, TRM in the first 90 days after transplantation occurred in 75% ($n = 22$) of those patients ($n = 30$) who had a cumulative toxicity score above the 95th percentile (score >2.34). By linear model testing the association of pre-transplant characteristics with cumulative toxicity, as shown in Table 5, we estimated that a higher score was significantly associated with transplantation from alternative donors ($P < 0.001$); age, diagnosis and phase at transplantation, and conditioning regimen did not reach statistical significance. According to the type of transplant, the mean cumulative toxicity score was 0.07 (s.d.: 0.76) in patients undergoing autologous transplantation, 0.23 (s.d.: 0.95) in those undergoing allogeneic transplantation from a compatible related donor, and 0.60 (s.d.: 1.25) from an alternative donor. According to the type of transplant, there was an increasing trend both in incidence of moderate and severe toxicity in the relevant organs (lungs, heart, liver, kidneys), and in early TRM, as shown in Table 6.

Table 4 Relationship between early organ toxicity and non-leukemic death within 90 days after transplantation. Results of the logistic model[a] ($n = 636$)

Organ	*Estimated coefficient*	*Relative risk (95% confidence interval)*	*P value (Wald)*
CNS	0.77	2.2 (0.8–5.5)	0.11
heart	2.21	9.1 (2.8–29.6)	<0.001
lungs	1.70	5.5 (2.3–13.0)	<0.001
liver	1.00	2.7 (1.4–5.3)	0.003
gut	–0.68	0.5 (0.2–1.2)	0.11
kidneys	1.02	2.8 (1.2–6.2)	0.01
bladder	0.65	1.9 (0.9–4.0)	0.09
mucosae	–0.33	0.7 (0.4–1.2)	0.22

[a]Calculation of the cumulative toxicity score. Based on this output, a score was calculated for each patient by the weighted sum of the toxicity codes, with the weights being the estimated coefficients. For example, a patient having either moderate or severe heart toxicity, with all others being absent or mild, would have a score of 2.21, while a patient having severe toxicity of both heart and lungs would have a score of 2.21 + 1.70 = 3.91.

Discussion

This prospective AIEOP study achieved its aims of assessing early toxicity and TRM in a pediatric population transplanted for AL in the last decade, and studying the relationship between early organ toxicity and TRM, and between pre-transplant characteristics and cumulative toxicity. Consistent with other reports, overall TRM was 18.9% (s.e.: 1.9), with a 3-year EFS of 45.2% (s.e.: 2.1); most events (76%) occurred within 3 months, and 97% within 1 year after transplant. As expected, TRM was lowest in the autologous setting (6.2%, s.e.: 1.7), intermediate in allogeneic transplants from compatible related donors (14.9%, s.e.: 2.1), and highest in transplants from alterna-

Table 5 Relationship between pre-transplant characteristics and cumulative toxicity. Results of the linear model ($n = 636$)

Variable	*Category*[a]	*Regression coefficient (s.e.)*	*P value*
Age	<3 years	—	
	3–9 years	–0.02 (0.13)	0.85
	⩾10 years	0.12 (0.13)	0.34
Diagnosis and phase	ALL I–IICR, and ANLL ICR	—	
	advanced ALL and ANLL	0.10 (0.09)	0.25
Type of transplant	autologous	—	
	allogeneic related compatible	0.14 (0.09)	0.14
	alternative donor	0.47 (0.12)	<0.001
Conditioning regimen	chemotherapy	—	
	TBI + 1 drug	–0.21 (0.11)	0.06
	TBI + > 1 drug	0.06 (0.10)	0.52

[a]The first category reported is the reference one.
s.e. = standard error; ALL = acute lymphoblastic leukemia; ANLL = acute non-lymphoblastic leukemia; CR = complete remission; TBI = total body irradiation.

Table 6 Morbidity and mortality according to type of transplant in patients reporting moderate or severe toxicity[a]

Organ	*Autologous (n = 216)*			*Allogeneic compatible related donor (n = 294)*			*Allogeneic alternative donor (n = 126)*		
	n	*(%)*	*Early deaths*	*n*	*%*	*Early deaths*	*n*	*%*	*Early deaths*
Lungs	10	(4.6)	1	14	(4.8)	9	11	(8.7)	10
Heart	4	(1.9)	2	7	(2.4)	4	8	(6.4)	8
Kidneys	3	(1.4)	1	20	(6.8)	8	25	(19.8)	13
Liver	17	(7.9)	1	48	(16.3)	15	20	(15.9)	14

[a]Absolute numbers (*n*), percentages (%), and early non-leukemic death of patients experiencing moderate or severe toxicity are reported for the four organs (heart, lungs, liver, kidneys) according to type of transplant.

tive donors (51.3%, s.e.: 4.7).[1–11] Most patients developed toxicity to the mucosae, which was severe in only 8% of the cases; other than mucosae, the sites of most frequent toxicity were gut and liver. Patients experiencing multiple toxicities had higher TRM. To some degree, the grading of toxicity was subjective, which could result in variability of registration among investigators. In an attempt to look for inter-center variability, we found that the association between toxicities and TRM was homogeneous, as neither adjustment per center changed the relative risk for each organ, nor was each center significantly different from the others. Results of the logistic model indicated that any moderate or severe toxicity, other than mucosal and gastrointestinal, was associated with early death, with significant and higher relative risks of 9.1, 5.5, 2.7 and 2.8, for heart, lungs, liver and kidneys, respectively. A trend showed that the relative risk of early death increased with the grade of toxicity. Isolated toxicity to mucosae or gut did not seem to increase the risk of death; even though most patients experienced mucosal damage, it was rarely lethal *per se*, if other more relevant toxicities were absent. The lack of data regarding duration of toxicity in one-third of the cases confirmed that registration of variables regarding toxicity can be complex. To rule out the possibility that the relevance of organ toxicity was lowered in a heterogeneous population, the analysis was repeated in the subset of allogeneic transplants, yielding consistent results. The lack of statistical significance of liver and lung toxicities in the subgroup of patients without aGVHD, as compared to the overall analysis, was probably attributable to the subgroup size; it also indirectly showed that liver and lung toxicity were more predictive of early death in patients who experienced aGVHD, than in those who did not. Bearman's requirement to separate toxicity from aGVHD and infections was difficult, and mostly concerned liver, gut and lung toxicities, since these are often concurrent and amplify each other, as shown by the doubling of TRM in patients experiencing severe aGVHD[23,26–30] and by the increase in probability of TRM of almost three and 12 times in the allogeneic setting, conventional and alternative, respectively, as compared to the autologous setting. Patients transplanted from alternative donors experienced the highest TRM in this series, partially explained by the significantly higher incidence of severe aGVHD; a possible explanation is that at the beginning of the 8-year interval, only patients with the worst prognosis were treated by alternative transplant approaches, which were not yet validated. More recent studies, also within the AIEOP, reported a lower TRM, possibly due to better patient selection, improvement in supportive therapy, and ultimately potentiated immunosuppression, mostly needed in the unrelated setting.[34] The 'cumulative toxicity', as usual clinical prognostic scores, consisted of linear combinations of covariates 'weighted' by each organ coefficient estimated by the regression model.[35–37] The cumulative toxicity score was 'low' in most patients, consistent with the conception of the model, in a population with a relatively low TRM; if a subset of patients with a higher TRM was considered, such as patients transplanted from unrelated or mismatched related donors, the mean score was higher. The good prediction of TRM by the cumulative toxicity score was due to the design of the model, consistent with the clinical observation that patients who experience multiple and severe toxicities often die.[13] By the time toxicities are reported, it is often too late to change the course of the transplant. Prediction of the prognostic score before transplant was then explored, in order to direct choice of treatment, when the decision-making process is still possible and alternatives could be adopted. Such choices included chemotherapy alone or less toxic conditioning regimens.[4,13,38,39] The purpose of the linear model was to test whether pre-transplant characteristics were predictive of cumulative toxicity. As expected, type of transplant and donor proved to be significantly associated with cumulative toxicity ultimately related to TRM; diagnosis and phase of disease were associated with TRM only if the variable of the stronger effect of type of transplant was excluded, or if patients transplanted from other than compatible related donors were removed from the analysis (data not shown). The cumulative toxicity in this population was not significantly increased by any of the categories of conditioning regimen considered, which implies that irradiation apparently did not increase fatal toxicity, but does not exclude the possibility that early toxicity in 'non-crucial' organs, such as mucosae or gut, and long-term toxicity were increased by TBI, as opposed to chemotherapy only regimens. Prediction of TRM may overcome linear algorithm and involve many factors other than toxicity, such as possible pre-existing toxicities, or infections, which were the most frequent causes of non-leukemic death, mostly in patients with aGVHD.[1]

In conclusion, our prospective multicenter study achieved its goals of assessing early toxicity and TRM and their relationship in children transplanted for acute leukemia. Heart, lungs, liver and kidneys were identified as the

most relevant organs, in which toxicity was significantly associated with TRM. The cumulative toxicity score, calculated as a risk factor for TRM, was partially predicted by type of transplant and donor, among the pre-transplant characteristics we considered; the highest risk factor associated with TRM was a transplant from an unrelated or partially mismatched related donor. Efforts should be directed towards identifying new GVHD prophylaxis and supportive strategies in order to prevent toxicity and improve ultimate outcome, especially in patients undergoing allogeneic transplantation from alternative donors. Finally, for high risk patients, alternative options, such as 'reduced-intensity' or 'non-myeloablative' conditioning regimens, could be explored in multicenter trials, to reduce the toxicity and mortality induced by conditioning regimens.

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