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A risk factor analysis of outcomes after unrelated cord blood transplantation for children with Wiskott-Aldrich syndrome

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ABSTRACT

Wiskott-Aldrich syndrome is a severe X-linked recessive immune deficiency disorder. A scoring system of Wiskott-Aldrich syndrome severity (0.5-5) distinguishes two phenotypes: X-linked thrombocytopenia and classic Wiskott-Aldrich syndrome. Hematopoietic cell transplantation is curative for Wiskott-Aldrich syndrome; however, the use of unrelated umbilical cord blood transplantation has seldom been described. We analyzed umbilical cord blood transplantation outcomes for 90 patients. The median age at umbilical cord blood transplantation was 1.5 years. Patients were classified according to clinical scores [2 (23%), 3 (30%), 4 (23%) and 5 (19%)]. Most patients underwent HLA-mismatched umbilical cord blood transplantation and myeloablative conditioning with antithymocyte globulin. The cumulative incidence of neutrophil recovery at day 60 was 89% and that of grade II-IV acute graft-*versus*-host disease at day 100 was 38%. The use of methotrexate for graft-*versus*-host disease prophylaxis delayed engraftment (*P*=0.02), but decreased acute graft-*versus*-host disease (*P*=0.03). At 5 years, overall survival and event-free survival rates were 75% and 70%, respectively. The estimated 5-year event-free survival rates were 83%, 73% and 55% for patients with a clinical score of 2, 4-5 and 3, respectively. In multivariate analysis, age <2 years at the time of the umbilical cord blood transplant and a clinical phenotype of X-linked thrombocytopenia were associated with improved event-free survival. Overall survival tended to be better in patients transplanted after 2007 (*P*=0.09). In conclusion, umbilical cord blood transplantation is a good alternative option for young children with Wiskott-Aldrich syndrome lacking an HLA identical stem cell donor.

Introduction

Wiskott-Aldrich syndrome (WAS) is a severe X-linked recessive immune deficiency disorder caused by mutations in the gene encoding for Wiskott-Aldrich syndrome protein (WASP), a key regulator of actin polymerization signaling and cytoskeletal reorganization in hematopoietic cells.[1-3] A mutation in *WASP* results in a broad spectrum of clinical manifestations ranging from the relatively mild X-linked thrombocytopenia (XLT) to the classic WAS phenotype characterized by microthrombocytopenia, immunodeficiency, eczema, and high susceptibility to lymphoproliferative tumors and autoimmune diseases.[2,4,5] A simple scoring system on a scale from 0.5 to 5 was introduced to differentiate XLT from classic WAS patients based on the severity of the clinical phenotype (*Online Supplementary Table S1*).[6]

XLT patients (score <3) have excellent overall survival (OS), but also have a high probability of severe disease-related complications.[7] In contrast, the classic WAS (score ≥3) usually leads to death in early childhood or adolescence, despite advances in clinical care, with a median life expectancy of only 15 years.[6,8,9]

Currently, the only proven curative therapy for patients with WAS is hematopoietic stem cell transplantation (HSCT).[6,10] Various series of HSCT from HLA-matched related donors have consistently resulted in survival rates above 80% for patients with WAS.[11-15] In the absence of a matched related donor, the OS reported after matched unrelated HSCT has been around 70%.[11] In the last 20 years, unrelated donor umbilical cord blood transplantation (UCBT) has become an option for patients lacking an HLA-matched donor.[16] To date, there are only few reports on outcomes after UCBT for patients with primary immune deficiencies,[17-19] and they include only a few patients with WAS;[18,20-22] furthermore, none of the studies has analyzed factors associated with outcomes after UCBT. We, therefore, conducted a collaborative, multicenter, retrospective risk factor analysis of patients with WAS reported to Eurocord. A total of 90 UCBT recipients met the criteria and were included in the study.

Methods

Data collection

This retrospective analysis is based on data reported to the European Blood and Marrow Transplantation group (EBMT) and/or Eurocord from European and non-European transplant centers through a standardized questionnaire that included information on patients, donors, diseases, and transplant outcomes. Missing information was requested in the form of a Microsoft Excel file listing transplants performed by each center along with key data extracted from the Eurocord-EBMT databases. In addition, data from Duke University Medical Center (USA), the Federal University of Parana (Brazil) and the Pontifical Catholic University of Chile were obtained from the respective centers. Recipients' parents or legal guardians gave informed consent for HSCT according to the Declaration of Helsinki. Eurocord and the Working Party of Inborn Errors of the EBMT approved this study.

Inclusion criteria

The inclusion criteria for the study were: (i) patients transplanted for WAS before December 31, 2013, and reported to Eurocord; (ii) first allogeneic unrelated HSCT.

Patients were excluded from the study if the diagnosis of immune deficiency was not specified or if transplants were performed with a cord blood unit that was expanded, combined with other sources of hematopoietic stem cells, or injected intrabone.

Endpoints and definitions

The primary endpoint was: event-free survival (EFS), defined as survival from transplantation to last contact without any of the following events: autologous reconstitution (defined by documentation of <5% donor-derived engraftment), graft failure (defined as a lack of neutrophil recovery or transient engraftment of donor cells after transplantation, and/or a requirement for a second transplant) and death. All surviving patients were censored at the date of last contact.

Other endpoints reported included: (i) OS, defined as the time from transplantation to death from any cause; (ii) cumulative incidence of neutrophil engraftment, defined as the first day of achieving a neutrophil count of ≥$0.5x10^9$/L for 3 consecutive days with evidence of donor hematopoiesis; (iii) cumulative incidence of platelet engraftment, defined as the first of 3 consecutive days after HSCT with a platelet count ≥$20x10^9$/L without platelet transfusions for at least 7 days; (iv) graft failure: primary failure defined as the neutrophil count never reaching $0.5x10^9$/L or evidence of autologous reconstitution; secondary graft failure defined as reaching a neutrophil count of $0.5x10^9$/L after transplantation, but experiencing a subsequent, non-transitory, decrease, or loss of donor chimerism; and (v) the incidence of acute and chronic graft-*versus*-host disease (GvHD). Acute GvHD grade II-IV was diagnosed and graded according to published criteria.[23] Chronic GvHD was also graded according to standard criteria[24] and evaluated in patients who survived at least 100 days with sustained engraftment.

Myeloablative conditioning was defined as conditioning including an intravenous busulfan total dose of more than 6.4 mg/kg or an oral dose greater than 8 mg/kg/day, or treosulfan >36 mg/m^2 for infants (less than 12 months old) and >42 mg/m^2 for others. Other regimens were considered reduced intensity conditioning.

Donor-recipient HLA matching was defined considering low resolution typing for HLA class I (A and B) and high resolution typing for HLA class II (DRB1). Donor-recipient chimerism was reported on the basis of available data during the first 100 ± 30 days, 1 year ± 30 days after UCBT and at the last chimerism evaluation. Full donor chimerism was defined as the presence of ≥95% donor-derived hematopoietic cells, mixed-chimerism as 5% to 94% of these cells and autologous recovery if <5%.

The patients' immunophenotype ($CD3^+CD4^+$ and $CD3^+CD8^+$ T-lymphocytes; $CD19^+$ B-lymphocytes) was determined 100 ± 30 days, 1 year ± 30 days and at the last assessment reported after UCBT. As normal values in childhood vary considerably with age, absolute numbers of $CD4^+$, $CD8^+$ and $CD19^+$ cells were related to age-specific normal values.[25,26] Immune recovery was defined as being alive with neutrophil engraftment and achieving absolute numbers of $CD4^+$, $CD8^+$ and $CD19^+$ cells within the age-related normal values, as shown in *Online Supplementary Table S2*.

Statistical analysis

To analyze risk factors for outcomes, we considered factors related to the patient (median age at diagnosis, median age at transplant, median weight at time of transplantation, gender, pre-transplant cytomegalovirus serology status, Lansky score), disease [pre-transplant information on: infections, severe thrombocytopenia (platelets <$20x10^9$/L), number of platelet transfusions, platelet abnormalities, history of severe bleeding, splenectomy, presence of eczema, autoimmunity, malignancy, congenital neutropenia, clinical phenotype, median interval from date of birth to diagnosis, median interval from diagnosis to transplant], cord blood unit (HLA matching, median numbers of total nucleated cells and $CD34^+$ cells collected and infused), and the transplant (year of transplantation, use of reduced intensity or myeloablative conditioning, type of GvHD prophylaxis).

Cumulative incidence curves were calculated for neutrophil and platelet engraftment, and acute and chronic GvHD in a competing risk setting, with death as a competing event.[27] Gray test was used for univariate comparisons. Probabilities of EFS and OS were calculated using the Kaplan-Meier estimate; the two-sided log-rank test was used for univariate comparisons. Multivariate analyses were performed using the Cox proportional hazard regression model[28] for EFS and OS, and the proportional sub-distribution hazard regression model of Fine and Gray for acute and chronic GvHD, and neutrophil and platelet engraftment. Variables that reached a *P*-value of 0.10 in the univariate analysis, and other relevant factors such as HLA matching and cell dose, were included in the initial models and variables were eliminated one by one in a stepwise fashion in order to retain only those variables that reached a *P*-value of 0.05 in the final model. *P*-values were two-sided. Statistical analyses were performed using SPSS (Inc., Chicago, IL, USA) and S-Plus (MathSoft, Inc., Seattle, WA, USA) software packages.

Results

Characteristics of the patients, donors and transplants

Ninety patients with a clinical diagnosis of WAS who underwent UCBT between 1996 and 2013 in 33 centers from 20 countries met the eligibility criteria for the study. The baseline characteristics of the patients, donors and transplants are shown in Table 1. Disease severity before transplantation was expressed as a WAS score of 2 to 5. Eighteen patients (23%) had a WAS clinical score of less than 3 at the time of UCBT, indicating that they had not experienced any of the following: severe infections, difficult-to-treat eczema, autoimmunity, or malignancy. The majority of patients (n= 61, 77%) had severe clinical features of the disease at the time of transplantation, including 52 patients with a history of recurrent and/or severe infections. Seven patients had a history of autoimmune disorders and two had a history of Epstein-Barr virus-associated lymphoproliferative disease.

Four patients were splenectomized before UCBT, of whom two had a platelet count <20x10⁹/L at the time of transplantation. All of the splenectomized patients had the classic WAS clinical phenotype and three experienced severe infection before UCBT.

Data on WAS gene mutations were available for 39 patients (43%). Almost equal proportions of patients carried nonsense, missense and deletion mutations.

The median age at transplantaion was 1.48 years (range, 4.8 months – 14.25 years). Only nine patients were more than 5 years old at the time of UCBT. Most patients (76%) had a good performance status at the time of UCBT (Lansky score >80%).

The vast majority of children were conditioned with a busulfan-containing myeloablative regimen (97%), mainly busulfan/cyclophosphamide (76%). Three patients received reduced-intensity conditioning, two of them due to severe infection at the time of UCBT. Most patients received antithymocyte globulin (n=79). All patients who received GvHD prophylaxis received a calcineurin inhibitor-containing regimen, including either cyclosporine A (in most cases)

Table 1. Baseline characteristics of the patients, disease, donors and transplants.

	N =90 (%*)	Median (range)
Patients' characteristics		
Gender (male/female)	88/2	
Weight, kg	10.25 (5-51.7)	
Age at diagnosis, years		0.32 (0-8.3)
Time interval diagnosis- UCBT, years		1.05 (0.06-13.05)
Age at transplantation, n.		1.48 (0.40-14.25)
≤5 years	81	
> 5 years	9	
CMV status before UCBT, n (%)		
Seropositive	47(70)	
Seronegative	20 (30)	
Disease characteristics, n (%)		
WAS clinical phenotype at UCBT		
Severe infections	52 (68)	
Eczema		
Mild	19 (28)	
Moderate	35 (51)	
Severe	14 (21)	
Severe thrombocytopenia (<20x10⁹cells/L)	43 (70)	
Microthrombocytopenia	22 (51)	
Life-threatening bleeding	41 (59)	
Malignancies	2 (4)	
Autoimmunity	7 (13)	
Congenital neutropenia	9 (19)	
WAS score		
2	18 (23)	
3	24 (30)	
4	18 (23)	
5	19 (24)	
Splenectomy before UCBT	4 (5)	
Donors' characteristics, n (%)		
HLA-matching		
6/6	11 (12)	
5/6	52 (58)	
4/6	25 (28)	
3/6	1	
Cell dose		
Collected NC ($x10^7$/kg)		7.5 (0.2-3)
Collected $CD34^+$ ($x10^5$/kg)		3.03 (0.03-35)
Transplant characteristics		
Year of transplantation		2007 (1996-2013)
Conditioning regimen		
Myeloablative	87 (97)	
Cy/Bu	67	
Cy/Bu+others	8	
Bu/Fluda	6	
Fluda/Treo 42	5	
Cy/Hydroxyurea	1	
Reduced intensity	3	
Cy/Bu	1	
Fluda/Melph ± Treo 36	1/1	
GvHD prophylaxis		
CNI/prednisolone	49 (54)	
CNI/methotrexate		17 (20)
CNI/mycophenolate mofetil		11 (12)
CNI	9 (10)	
CNI/others	3 (3)	
Not given	1 (1)	
Serotherapy		
Anti-T serotherapy (before day 0)	79 (89)	
Monoclonal antibody	2 (2)	
Not given	8 (9)	

*Percentage of evaluable cases. UCBT: umbilical cord blood transplantation; CMV: cytomegalovirus; WAS: Wiskott-Aldrich syndrome; NC: nucleated cells; MAC: myeloablative conditioning regiment; Cy,: cyclophosphamide; Bu: busulphan; Fluda: fludarabine; Treo: treosulphan; GvHD: graft-*versus*-host disease; CNI: calcineurin inhibitor.

or tacrolimus. The median numbers of total nucleated cells and CD34$^+$ cells infused were 6.8x10^7 cells/kg and 3.04x10^5/kg (pre-cryopreservation counts), respectively.

Neutrophil and platelet recovery

Eighty patients (89%) achieved neutrophil engraftment, with a median time to engraftment of 21 days (range, 9-54). The cumulative incidence of neutrophil engraftment was 70% and 89% at days 30 and 60, respectively. Ten (11%) patients did not achieve neutrophil engraftment. Of the patients who failed to engraft, four received a second transplant and were alive at last follow-up; six did not receive a second HSCT and died.

Multivariate analysis showed that use of methotrexate in GvHD prophylaxis [hazard ratio (HR)=0.55, 95% confidence interval (95% CI): 0.32-0.93; *P*=0.02)] was associated with a lower cumulative incidence of neutrophil engraftment.

At day 180, the cumulative incidence of platelet engraftment was 75%, with a median time to engraftment of 45 days (range, 11-224). Platelet engraftment in the four splenectomized patients seemed faster, occurring at a median time of 35 days. Due to the small number of splenectomized patients, it was not possible to confirm whether this procedure has a real impact on the engraftment rate. In univariate analysis, the only risk factor associated with a lower cumulative incidence of platelet engraftment was age more than 2 years (67% *versus* 79% for younger patients, *P*=0.03). Multivariate analysis confirmed that age was independently associated with platelet engraftment (HR=0.34, 95% CI: 0.16-0.73; *P*=0.005).

Chimerism and immune recovery

Chimerism data were available for 66 (86%) out of 77 evaluable patients at 100 (±30) days after UCBT, 50 (80%) out of 63 patients at 1 year (±30 days) after UCBT, and 51 (82%) out of 62 patients at the last assessment. At day 100, 68% of patients had full donor chimerism and 32% had mixed chimerism; at 1 year, these values were 76% and 24%, respectively while at the last assessment they were 80% and 20%, respectively. In 12 cases, mixed chimerism became full donor reconstitution in a further assessment, and two patients who, initially, had full donor reconstitution became stable mixed chimera at their last assessment.

Information on the absolute number of CD3$^+$/4$^+$, CD3$^+$/8$^+$ and CD19$^+$ lymphocytes at 100 ± 30 days, 1 year ± 30 days and at the latest assessment after UCBT was available for 29, 25 and 25 of the patients who were alive with neutrophil engraftment at the specific time-points, respectively. In this subset analysis, 31 (67%) out of 46 patients achieved immune recovery. The median time between UCBT and the first reported immune recovery testing was 12 months; 11 patients achieved immune recovery within the first 12 months after transplantation; the earliest confirmed immune recovery was reported 4 months after UCBT. Fifteen patients did not achieve immune recovery. Of these, 13 patients were being treated with immunosuppressive agents due to acute GvHD; the remaining two patients, who had no history of GvHD, had mixed chimerism results.

Acute and chronic graft-*versus*-host disease

Acute GvHD grade II-IV was observed in 35 patients: 22 had grade II (24%), 8 had grade III (8%), and 5 had grade IV (6%). The cumulative incidence of acute GvHD grade II-IV at day 100 was 38%. In univariate analysis, none of the factors analyzed was significantly associated with an increased risk of grade II-IV GvHD. However, in multivariate analysis, the use of methotrexate for GvHD prophylaxis was associated with a decreased incidence of grade II-IV GvHD (22% *versus* 42%) (HR=0.34, CI 95%: 0.12-0.91; *P*=0.03). The cumulative incidence of chronic GvHD at 5 years was 17% (n=15; 6 extensive and 9 limited cases). In univariate analysis, the cumulative incidence of chronic GvHD decreased after 2007 (25% *versus* 3%, *P*<0.01). Chronic GvHD also decreased for patients receiving a total nucleated cell dose lower than 6.8x10^7/kg, (23% *versus* 7%, *P*=0.03). In multivariate analysis, none of the risk factors studied was significantly associated with an increased risk of chronic GvHD.

Overall survival, event-free survival and causes of death

The probabilities of OS and EFS at 5 years were 75±5% and 70±5%, respectively. Table 2 shows the univariate analysis of risk factors for OS and EFS. The risk factors associated with worst OS in multivariate analysis (Table 3) were: age over 2 years at UCBT (HR=2.61, 95% CI: 1.1-6.16; *P*=0.02) and clinical score >2 (HR=4.49, 95% CI: 1.02-19.78; *P*=0.04)] (Figures 1 and 2). There was a trend to improved OS in patients transplanted after 2007 (HR=2.27, 95% CI: 0.86-5.98; *P*=0.09) (Figure 3). In multivariate analysis for EFS, older children (more than 2 years) at UCBT also had a significantly worse prognosis (HR=2.47, 95% CI: 1.1-5.52; *P*=0.02)]. Sixty-seven patients were alive at the last assessment, with a median follow-up of 5 years (range, 0.25-17). Twenty-three (25%) patients had died. Table 4 shows causes of death less than and more than 100 days after UCBT, according to WAS score. Infection-related deaths were commonly observed among patients with all disease scores, and infection was the main cause of death, especially before day 100.

Discussion

This multicenter, retrospective study on UCBT recipients with WAS confirms that, for most patients, HSCT using HLA-matched or -mismatched cord blood cells can cure and prevent the long-term, life-threatening complications associated with WAS. Outcomes of patients with WAS who do not undergo HSCT remain poor, with the mean age at death being 20 years in previous reports[9] and with increasing risk of malignancies with age. Several groups[11,15,16] reported successful HSCT results with an OS of up to 88% when using a "gold standard donor".[14] In the absence of a matched related donor, other groups have reported the successful use of matched unrelated donors with 71% OS.[11,29,30] but with higher risks of acute and chronic GvHD. Unfortunately, many patients do not have an available matched unrelated donor and, therefore, other donor sources for transplantation have been investigated, such as T-cell-depleted haploidentical HSCT and umbilical cord blood HSCT. The results after haploidentical HSCT with TcRαβ/CD19-depletion for patients with primary immunodeficiency currently seem promising.[31] On the other hand, UCBT is still attractive because of the naïvety of the stem cells, the lower HLA matching requirements, and easy availability (compared to matched related and matched unrelated donors) and decreased GvHD (compared to haploidentical HSCT).[32]

We conducted this risk factor analysis for WAS, a rare disease, using retrospective-registry-based data. The limitations of our study are mainly due to some missing data related to the disease and the long inclusion period with changes in cord blood unit selection and better supportive care in more recent years. Despite these limitations, the study remains noteworthy, as it is the largest series of children with WAS treated with UCBT.

We were able to identify two main factors associated with EFS and OS after UCBT: age at UCBT and clinical disease score (Figures 1 and 2). EFS and OS were significantly improved when transplantation was performed before 2 years of age, with almost 80% of these young patients being cured. This finding supports the need for early referral for transplantation in infants diagnosed with WAS. We could speculate that older children could have more previous complications before UCBT, which, in turn, could affect outcomes. However, in patients with available data, we determined that OS and EFS were not associated with number of previous infections, Lansky score, severity of eczema, thrombocytopenia, number of previous platelet transfusions, autoimmunity or congenital neutropenia.

Clinical score was also a prognostic factor. As expected, patients with XLT (score <3) had better OS and EFS than patients with other clinical phenotypes. XLT patients have, historically, excellent OS without transplantation in contrast to patients with classic WAS. However, EFS in XLT patients seems to worsen over time.[33,34] Data from the XLT registry showed an EFS of 74% at 15 years, decreasing to 56% by 30 years, without subsequent transplantation. There is currently no consensus on the indications for HSCT in XLT and the decision regarding transplantation for such patients has to be made on an individual basis.[7] In our cohort, 23% of the patients were classified as having score 2, and there were no patients with a score of 0.5 or 1 (Table 1), showing that some degree of severity was present to justify the transplantation. We found that patients with a clinical score of 3 seemed to have worse OS and EFS probabilities, although we were not able to draw definitive conclusions because of the small number of patients in the groups.

The most frequent cause of death after UCBT was infection. The majority of patients in our series received anti-thymocyte globulin as part of their conditioning regimen, which may explain the high number of infection-related deaths observed.[35] Infections are commonly seen after UCBT due to delayed engraftment and impaired immune recovery mainly when anti-thymocyte globulin is used before UCBT.[36]

We were able to analyze immune recovery in a subset of patients. We found that 67% of the 46 patients with available information achieved immune recovery. The median time between UCBT and the first test reporting immune recovery was 12 months, and 11 patients achieved immune recovery within 12 months of transplantation. These results seem comparable to those regarding immune recovery usually observed after UCBT.[26] However, due to the retrospective nature of our analysis, we were unable to collect details on intravenous immunoglobulin use or vaccine-specific antibody responses; the immune recovery results reported here should, therefore, be taken with caution. Most patients who did

Table 2. Probability of 5-year overall and event-free survival after umbilical cord blood transplantation for Wiskott-Aldrich syndrome.

Variable	N	5 years OS % (95%CI)	*P*	5 years EFS % (95%CI)	*P*
Patients' characteristics					
Age at UCBT					
≤ 2 years	60	83 (71-91)	0.027	78 (67-86)	0.05
> 2 years	30	58 (41-74)		55 (38-71)	
CMV status					
negative	19	77 (54-91)	0.9	73 (51-88)	0.9
positive	47	80 (66-89)		74 (59-85)	
Weight at UCBT					
<10 kg	45	89 (76-96)	0.002	82 (68-91)	0.02
≥ 10 kg	45	60 (44-74)		58 (42-72)	
Splenectomy					
Yes	4	50 (15-85)	0.2	50 (15-85)	0.33
No	75	76 (42-93)		72 (61-81)	
Lansky score					
<90%	19	67 (44-84)	0.63	57 (34-77)	0.2
≥ 90%	59	76 (63-86)		74 (61-84)	
Disease characteristics					
Clinical score					
2	18	89 (69-97)	0.03	83 (61-94)	0.18
3	24	61 (41-77)		55 (35-75)	
4	18	78 (55-91)		72 (50-88)	
5	19	79 (57-91)		74 (52-88)	
UCBT characteristics					
HLA-match					
6/6	11	71 (41-90)	0.9	61 (33-83)	0.75
5/6	53	76 (63-86)		72 (59-82)	
4/6 or 3/6	26	72 (52-86)		69 (50-83)	
ABO-compatibility					
no incompatibility	36	70 (47-86)	0.8	72 (55-850	0.9
minor incompatibility	17	76 (58-88)		70 (47-86)	
major incompatibility	29	70 (49-85)		70 (51-84)	
Year of UCBT					
before 2007	51	69 (54-81)	0.13	65 (51-77)	0.2
after 2007	39	83 (66-93)		78 (62-51)	
Time from diagnosis to UCBT					
< 7 months	23	91 (73-97)	0.14	86 (68-95)	0.18
7-13 months	22	80 (56-93)		76 (53-90)	
>13-19 months	22	72 (50-87)		68 (47-83)	
> 19 months	22	58 (37-77)		54 (34-73)	
Conditioning regimen					
Cy/Bu/ATG	25	83 (64-93)	0.2	83 (60-91)	0.3
others	65	72 (60-81)		67 (55-77)	
GvHD prophylaxis					
methotrexate	18	74 (63-83)	0.7	70 (57-80)	0.7
others	72	78 (54-91)		72 (48-88)	
Cell doses					
TNC collected					
< $7x10^7$/kg	43	72 (57-83)	0.43	67 (52-79)	0.4
≥ $7x10^7$/kg	42	77 (61-88)		73 (58-84)	
TNC infused					
< $6x10^6$/kg	43	77 (61-88)	0.8	74 (59-85)	0.5
≥ $6x10^6$/kg	42	72 (57-83)		65 (49-79)	
$CD34^+$collected					
< $3x10^6$/kg	34	68 (51-81)	0.2	62 (46-76)	0.12
≥ $3x10^6$/kg	33	78 (59-90)		75 (57-87)	
$CD34^+$ infused					
<$3x10^5$/kg	34	66 (52-78)	0.11	74 (59-85)	0.14
≥$3x10^5$/kg	33	86 (64-95)		75 (57-87)	

UCBT: umbilical cord blood transplantation; OS: overall survival; EFS: event-free survival; CI: confidence interval; CMV,: cytomegalovirus; Cy: cyclophosphamide; Bu: busulphan; ATG: anti-thymocyte globulin; GvHD: graft-*versus*-host disease; TNC: total nucleated cells.

not achieve immune recovery were treated with immunosuppressive agents due to acute GvHD, which may explain our findings.

Mixed chimerism is an undesirable outcome following HSCT for WAS since it may be associated with lymphopenia, autoimmunity and thrombocytopenia.[16] In our series, chimerism data were available for 86% of the patients at 3 months, 12 months and the last assessment. Full donor chimerism was observed in 68%, 76% and 80% of the patients at these timepoints, respectively. In a recent study of chimerism in 194 HSCT recipients with WAS, 72.1% of patients achieved full and stable donor chimerism.[16] In this study it was also shown that mixed chimerism affects the myeloid compartment (16.5% of

Table 3. Multivariate analysis for overall survival and event-free survival.

	HR	95% CI	*P*
Overall survival			
Older than 2 years	4.49	1.02-19.78	0.04
WAS score more than 2	2.61	1.1-6.16	0.02
UCBT after 2007	2.27	0.86-5.98	0.09
Event-free survival			
Older than 2 years	2.47	1.1-5.52	0.02
WAS score more than 2	3.13	0.9-10.87	0.07
UCBT after 2007	1.68	0.7-4.04	0.24

HR,: hazard ratio; CI: confidence interval; WAS,: Wiskott-Aldrich syndrome; UCBT: umbilical cord blood transplantation.

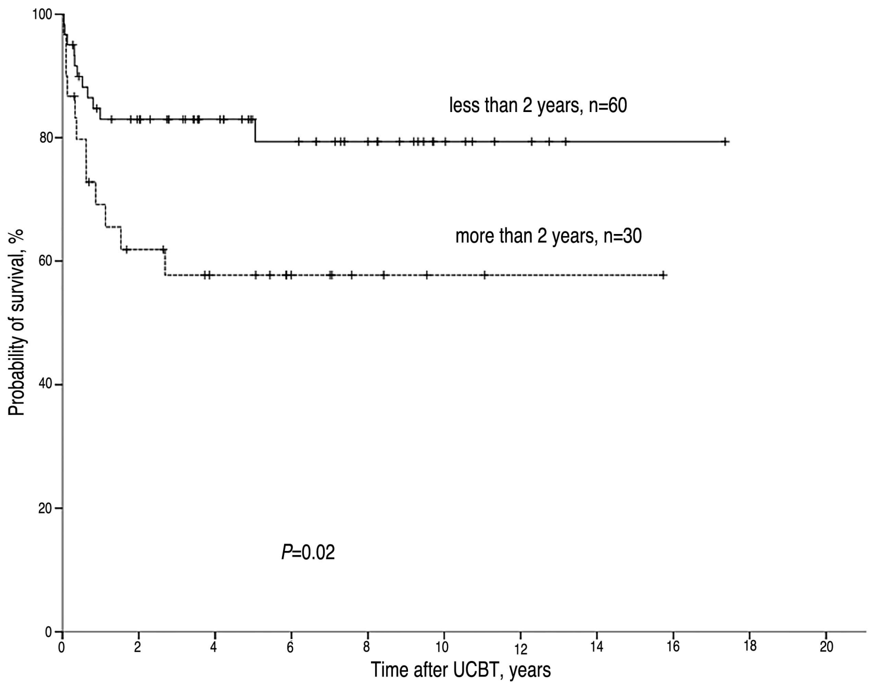

Figure 1. Probability of overall survival after umbilical cord blood transplantation for Wiskott-Aldrich syndrome according to age.

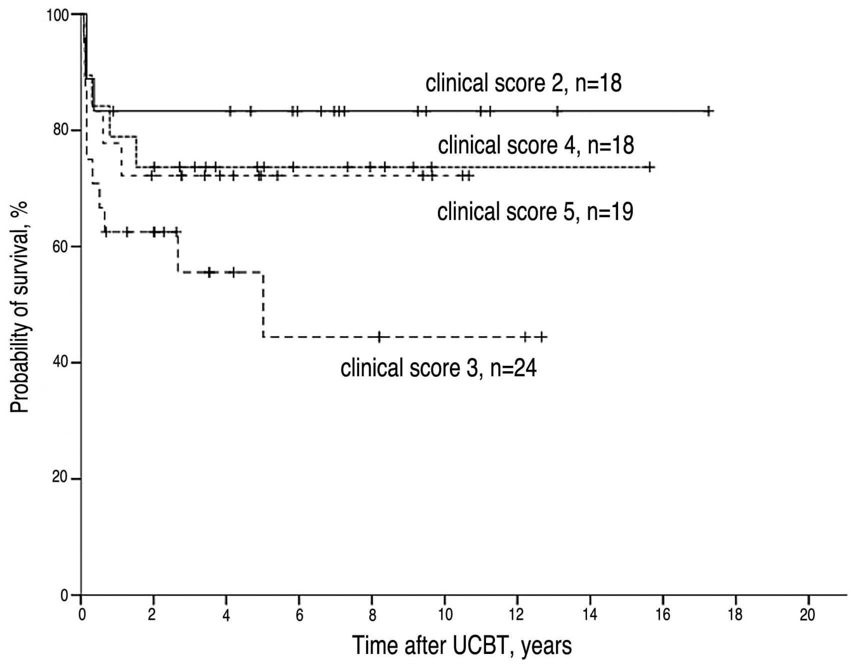

Figure 2. Probability of event-free survival after umbilical cord blood transplantation for Wiskott-Aldrich syndrome according to clinical score.

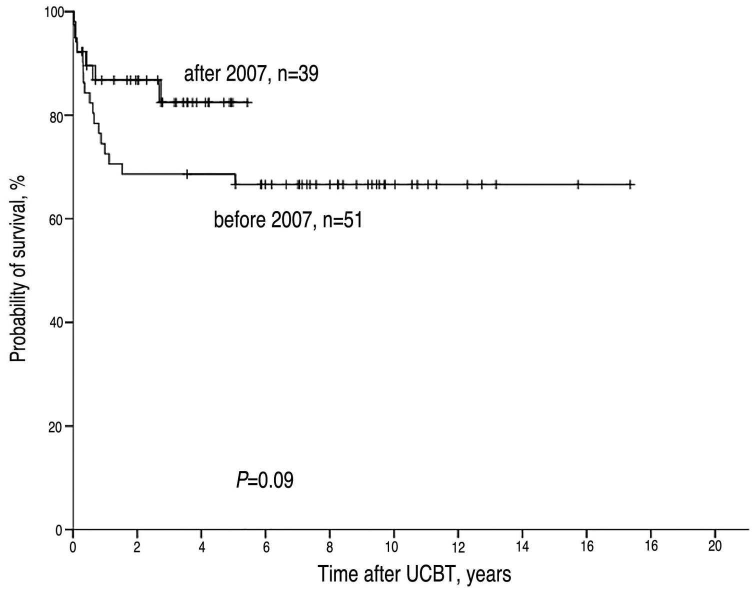

Figure 3. Probability of overall survival after umbilical cord blood transplantation for Wiskott-Aldrich syndrome according to year of transplantation.

Table 4. Primary causes of death before and more than 100 days after umbilical cord blood transplantation for Wiskott-Aldrich syndrome.

Primary causes of death	WAS clinical score					Total number
	2	3	4	5	Unknown	
N deaths/N total	2/18	11/24	4/18	4/19	2/11	
Before 100 days	**1**	**5**		**1**		**7**
Viral infection		3				3
Fungal infection	1			1		2
Bacterial infection		1				1
Unknown infection		1				1
After 100 days	**1**	**6**	**4**	**3**	**2**	**16**
Infection		5		1	1	7
GvHD	1			2		3
Multiorgan failure			2			2
Unknown			1		1	2
Interstitial pneumonitis			1			1
Hemorrhage		1				1

WAS: Wiskott-Aldrich syndrome; GvHD: graft-*versus*-host disease.

cases), followed by the B-cell compartment (7.4% of cases) and uncommonly the T-cell compartment (3.2% of cases). Unfortunately, in our series, we did not have data on lineage-specific chimerism; however, our results are comparable with the 72% full donor chimerism seen with other sources of hematopietic stem cells.

We were unable to identify cord blood donor-related factors associated with outcomes. Previously, the Eurocord group reported that for UCBT recipients with non-malignant disorders, a cell dose higher than $5x10^7$/kg and 6/6 or 5/6 HLA-matched grafts are associated with decreased mortality.[35] In our study, patients were transplanted with a median cell dose of $7.5x10^7$/kg and 70% received a 6/6 or 5/6 matched cord blood graft, which is in agreement with the recommendations for cord blood selection for patients with non-malignant disorders.

In conclusion, early referral for UCBT in patients with WAS is associated with better outcomes. New treatment strategies such as autologous gene-modified HSCT may overcome the disadvantages of graft rejection and GvHD after allogeneic HSCT. However, until these strategies become clinically available, UCBT remains a good alternative for patients lacking an HLA-matched donor.

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