Impact of Early Chimerism Status on Clinical Outcome in Children with Acute Lymphoblastic Leukaemia after Haematopoietic Stem Cell Transplantation

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Abstract

In this retrospective study we evaluated the clinical impact of very early chimerism after allogeneic haematopoietic stem cell transplantation (allo-HSCT) in children with acute lymphoblastic leukaemia (ALL). The study group included 38 boys and 18 girls. Very early chimerism was evaluated on days +7, +14, +21 and +28 after the transplant. Quantitative fluorescence polymerase chain reaction was used to evaluate chimerism. Overall survival (OS) and event-free survival (EFS) were 84% and 80% respectively. OS in group of 24 patients with complete donor chimerism on day +14 was 83% and it did not differ statistically compared to 32 patients with mixed chimerism on day +14 (OS was 84%). The donor type (matched unrelated) and sex (male; p = 0.02 on day +14, p = 0.01 on day +21, p = 0.02 on day 28), number of transplanted cell (above 4,47 x 106 kg; p = 0.036 on day +14) and serotherapy (without ATG; p = 0.05 on day +7, p = 0.016 on day +21, p = 0.023 on day 28) were statistically related to a high level of donor chimerism. Grades II-IV acute graft versus host disease were diagnosed in 23 patients, who presented level donor chimerism above 60% on day 7. The data presented in this study provides valuable insight to the analysis of very early chimerism in children with ALL treated with HSCT.

Introduction

Current chemotherapy regimens for acute lymphoblastic leukaemia (ALL) result in a remission in the majority of children with the disease. Despite remarkable improvement in the treatment of this malignancy, 20% of the children still relapse and their outcome remains poor [1]. Allogeneic haematopoietic stem cell transplantation (allo-HSCT) for these children has become a well-established treatment to control the disease [2]. The curative effect of allogeneic HSCT for acute leukemia is attributed to the graft-versus-leukemia effect produced by allogeneic immune cells, as well as intensive conditioning chemotherapy with or without radiotherapy [3].

It is well known that chimerism monitoring is an important diagnostic tool for assessing the risk of relapse after allo-HSCT in patients with malignant diseases, especially in those, who lack specific marker for tracking residual disease [4]. However, the significance of very early chimerism assessment before day +28, which is considered as the moment of the engraftment, is still unclear. The studies show that early analysis of T- and NK-cell chimerism can therefore be instrumental in risk assessment and therapeutic management of imminent graft rejection [5].

Investigations on the new methods for routine chimerism monitoring are very promising, however the current golden standard method of monitoring chimerism is Quantitative Fluorescence Polymerase Chain Reaction (QF-PCR), which not only determines the type of chimeras, but also determines the percentage of both donor's and recipient's cells [6, 7, 8]. The persistence or reappearance of recipient's cells after alloHSCT can indicate the presence of malignant cells or recurrence of recipient's haematopoietic cells or a combination of both [9].

The aim of this study was to analyse the dynamics of early chimerism after allogeneic HSCT in children with ALL and its role in the assessment of survival and event free survival. Furthermore, it analyses the

evolution of chimerism over the time and evaluates the impact of transplant variables on chimerism.

Materials And Methods

The research encompassed biological material (peripheral blood) derived from 56 consecutive children diagnosed with acute lymphoblastic leukaemia, who had undergone allogeneic haematopoietic stem cell transplantation at the Department of Paediatric Haematology, Oncology and Transplantology of Medical University in Lublin between 2002—2018. Patients' characteristics are summarized in Table 1.

All patients were conditioned according to contemporary EBMT guidelines [10]. Conditioning was myeloablative (MAC), standard regiments were based on fractionated total body irradiation (FTBI) or busulfane. In reduced toxicity conditioning (RTC) treosulfane was used instead of busulfane. Cyclosporine was used as a graft versus host disease (GvHD) prophylaxis. Matched unrelated transplant recipients received antithymocyte globulin (ATG) to prevent GvHD. Mismatched related transplant recipient received ex-vivo T-cell depleted graft. Engraftment was diagnosed when an absolute neutrophil count (ANC) of 500 or more was observed for 2 days.

Very early chimerism was evaluated from peripheral blood (PB) on days +7, +14, +21 and +28. Next samples were collected every 7–14 days until day +90. From day +100 chimerism was monitored according to the EBMT guidelines [9, 10]. Depending on clinical indications, chimerism was monitored irrespectively of the schedule time points.

The research material consisted of genomic DNA isolated from mononuclear peripheral blood cells. Peripheral blood was collected into anticoagulant (EDTA) containing tubes. The isolation of mononuclear cells (MNC) fraction was performed using Ficoll-Paque PLUS aqueous solution of 1.077 + 0.001 g/ml density, Amersham Biosciences. DNA isolations were performed with the QIAamp DNA Blood Mini Kit (Qiagen). The quantity of DNA was determined with a Nanophotometer (Thermo Fisher Scientific). According to the manufacturer's recommendations, it was used 1–2,5 ng matrix DNA with the commercial AmpFISTR NGM Plus Kit (Thermo Fisher Scientific). PCR was performed according to the manufacturer's instructions, which were as follows: 95°C for 11 min, followed by 28 cycles at 94°C for 20 sec, 59°C for 3 min, with an additional elongation step for 10 min at 60°C (ProFlex™ PCR System, Thermo Fisher Scientific) and final hold 4^0C. The following loci were amplified: D10S1248, vWA, D16S539, D2S1338 labelled with 5-FAM, Amelogenina X/Y, D8S1179, D21S11, D18S51 labelled with VIC, D22S1045, D19S433, THO1, FGA labelled with NED and D2S441, D3S1358, D1S1656 and D12S391 labelled with PET. Separation and detection of the PCR products were performed on a Genetic Analyzer 3500 (Thermo Fisher Scientific). Gene Mapper® ID-X software (Thermo Fisher Scientific) was used to automatically determine the size of the amplified fragments.

Statistical analyses were performed with STATISTICA 12.0 software. The Kaplan-Meier method was applied for survival analysis, and groups were compared by using log rank tests. P values < 0.05 were considered as significant.

The study was approved by the Ethics Committee of the Medical University of Lublin.

Results

The median follow-up of living patients was 4.58 years (1.00 - 15.79 years). Forty-eight patients (86%) are alive and in complete remission (in forty-five patients' complete donor chimerism was found, while recipient hematopoiesis was detected in three patients). Eight patients (14%) have died. Relapse occurred in five of them (9%), between 3 months and 4.5 years post HSCT. All of relapsed patients presented increasing recipient chimerism because of progression of leukemic disease. Three of relapsed patients achieved complete donor chimerism early at day +14. Three patients (5%) died due to transplant-related complication.

5-years overall survival (OS) and event-free survival (EFS) were 84% and 80% respectively. Analysis of early chimerism showed that on day +7 median donor chimerism level was 60%. The kinetics of early chimerism in the studied group of patients is presented in Figure 1.

OS and EFS were analysed for 24 patients with complete donor chimerism and 32 patients with mixed chimerism on day +14 after transplantation (Figure 2 and 3). OS was 84% for patients with complete donor chimerism and 83% for with mixed chimerism on day +14 after transplantation ($p = 0.9$). EFS was 80% for patients with complete donor chimerism and 79% for with mixed chimerism on day +14 after transplantation ($p = 0.59$).

In our cohort of patients, the donor type (matched unrelated), gender (male), number of transplanted cell (above 4,47 x 10^6 kg) and serotherapy (without ATG) were statistically related to high level of donor chimerism. The immunophenotypic of disease, age, recipient gender, stem cell source (PB/BM) and conditioning regimen had no impact on early chimerism (Table 2).

Grades II-IV acute graft versus host disease were diagnosed in 23 patients. No statistically significant effect was found of the level of donor chimerism above 80% achieved by patients before day +28 on the incidence of aGvHD ($p = 0.22$ on day 7; $p = 0.69$ on day 14; $p = 0.93$ on day 21; $p = 0.75$ on day 28). However, all patients with aGvDH presented level donor chimerism above 60% on day 7. The level of donor chimerism above 80% had no effect on chronic GvHD ($p = 0.05$ on day 7; $p = 0.93$ on day 14; $p = 0.85$ on day 21; $p = 0.27$ on day 28).

In two patients increasing recipient chimerism was found on day +21 and for that reason the cyclosporine was discontinued. These children have achieved complete donor chimerism on day +40 and +90 respectively. At the end of observation period both of them are alive and in complete remission 2.5- and 3.5-years respectively after HSCT with complete donor chimerism.

Discussion

The literature data suggest that chimerism analyses are routinely performed for the surveillance of engraftment. In recent years, these studies have become the basis for therapeutic intervention [11, 12]. The polymerase chain reaction of short tandem repeats (PCR-STR) method used in our study is a reliable tool, standardized in chimerism laboratory and well described in literature as the routine method for the assessment of post-transplant chimerism [13, 14]. The previous literature data suggested that serial mixed chimerism analysis in patients with acute leukemias at the short time intervals by PCR provides a reliable and rapid screening method for early detection of relapse [15]. Based on its limited sensitivity to detect minor cell population of about 1%, monitoring of chimerism in the whole blood is not suitable to serve as the minimal residual disease (MRD) marker. For the assessment of MRD, other techniques should be used [16].

Relapse after transplantation is a major cause of treatment failure in pediatric patients with ALL. Barrios M et al. [17] presented results which indicate that sequential determination of chimerism allows to predict relapse and death after HSCT for acute leukaemia. Patients with increasing mixed chimerism (IMC) showed a significantly higher ($p < 0.001$) rate of relapse (93.1%) and death (89.7%) in comparison to both those with complete chimerism (CC) (29.9% relapse, 44.1% dead) or decreasing MC (11.1% relapse, 44.4% dead). Relapse was found in 39.8% of analysed patients. The correlation between donor chimerism status and disease relapse after alloHSCT was investigated by Jiang Y et al [18]. 21.6% patients had recurrent disease. In the analysed group relapse was observed in 9% of patients who showed increasing recipient chimerism, although on day +14, they were complete chimeras. Five-year OS and 5-year event-free survival (EFS) were 62.07 ± 4.37% and 56.17 ± 4.38% respectively, for overall cohort of patients with ALL in the Pachon C et al. study [19]. In our study OS (83%) and EFS (84%) were higher than described in literature [19]. Three of our patients are alive with stable mixed chimerism (10% donor; 35% donor and 90% donor, respectively), which is in line with the observation of Levrat E. et al with very long-term stability of MC in patients with haematologic malignancies [20].

Lassaletta A et al. [21] presented results of the analysis of chimerism by the day 30 after peripheral blood progenitor cell transplantation. 27/39 patients showed CC by day 30 after HSCT, but median time to achieve CC was 15 days (range 8–750). In 15.4% patients, CC was never reached. On the day +7, median 61% of our patients presented very early complete donor chimerism, whereas on the day +14 median 90% patients had complete donor chimerism. Finally, 48/56 (86%) of our patients achieved complete donor chimerism.

Comparable to other studies, in our cohort no correlation between early donor chimerism and source of stem cell transplanted was found, but the number of transplanted CD34+ cells had a significant impact on patients' chimerism status [21, 22]. We observed that matched unrelated and male donor were connected with high level donor chimerism on day +7 and +14, similar to other report [23].

A relatively low-dose ATG is effective in acute GvHD prophylaxis, leading to promising survival rates in matched transplants [24]. Our results indicate that ATG is also effective, but connected to mixed chimerism in very early period after HSCT. In patients who received ATG as GvHD prophylaxis,

engraftment with complete donor chimerism was observed later than in patients without ATG (day +21 vs +14 respectively).

Conditioning regimen (myeloablative) did not affect early chimerism status, which is compatible with other reports [25, 27].

Some studies report, that the status of CC by day +30 was significantly related to the development of chronic GvHD. Patients who presented CC by day +30 had 80.8 +8%, probability of developing chronic GvHD, whereas the probability of patients with MC by day +30 was 48 +17% (P = 0.04) [21]. Mossalam G et al. [25] observed, that low donor chimerism in patients was connected with a reduced risk of chronic GvHD. Jaksch M et al. [26] found a significantly higher risk (p = 0.005) for developing aGvHD grades II-IV in patients with completed donor CD4+ T-cell chimerism day 7 after SCT together with patients who increased 50% or more in donor CD4+ T cells between days 7 and 10 after SCT. We did not observe correlation between early donor chimerism and aGvHD or cGvHD,perhaps due to the GvHD prophylaxis. The patients who developed cGvHD, presented on day +7 (p = 0.05) donor chimerism above 80%. To confirm this finding, the studies should be continued on a larger group of patients.

Horn B et al. [28] describe long term follow up of children with acute leukemia with early mixed chimerism-based posttransplant immunotherapy. Children receiving posttransplant immunotherapy achieved similar outcome to patients achieving full donor chimerism spontaneously. Rettinger E et al. added that the immunotherapy in the patients with mixed chimerism improves survival in childhood ALL and does not increase risk of acute GvHD [29]. In our cohort a successful therapeutic intervention was undertaken twice on the basis of chimerism measurements in the early posttransplant period (day +21).

Conclusion

The data presented in this study provides a valuable input on the analysis of the significance of very early assessment of chimerism in children with ALL treated with HSCT. Our findings suggest that early monitoring of chimerism after HSCT may be helpful tool in predicting transplant rejection and using successful therapeutic intervention. This study has its limitations, like no evaluation of the chimerism in lymphocyte subpopulations. The prospective observational studies and multicentre retrospective studies on larger groups of patients, including those diagnosed with different malignancies would allow to compare the results obtained for different groups of patients.

Declarations

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Conflict of interest statement

There is no conflict of interest to report.

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None. No founding to declare.

Authors' contribution

ML, AZP and KD are responsible to the conception and design of the study. AZP, JZ, AM and DG shared patients' clinical data and peripheral blood samples. ML and AM conducted laboratory work. ML, AZP and KD responsible for analysis and interpretation data. ML, KD and JRK prepared final manuscript for publication. This manuscript was reviewed and approved by all authors.

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Tables

Table 1. Characteristics of patients and transplantation data.

	Patients n=56 (100%)
Immunophenotype	
B ALL	31 (55%)
T ALL	25 (45%)
1 CR	26 (46%)
>2 CR	30 (54%)
Median age at transplant (range) years	9,04 (1,72-17)
Patient gender	
Male	38 (68%)
Female	18 (32%)
Donor type	
Matched related	25 (45%)
Matched unrelated	28 (50%)
Mismatched related	3 (5%)
Donor gender	
Male	31 (55%)
Female	25 (45%)
Stem cell source	
Bone marrow	47 (84%)
Peripheral blood	9 (16%)
Conditioning regimen	
Radiation-base	41 (73%)
Busulfan-based	2 (3%)
Reduced toxicity	13 (24%)
Serotherapy (ATG)	
YES	28 (50%)
NO	28 (50%)
Number of CD34+ cells (median 4,47) range (2 - 13,3 x 10^6/kg)	
< 4,47 x 10^6	35 (62%)
> 4,47 x 10^6	21 (38%)
aGvHD	23 (41%)
cGvHD	4 (7%)

B ALL - B-cell acute lymphoblastic leukaemia; T ALL - T-cell acute lymphoblastic leukaemia; CR - complete remission; ATG - antithymocyte globulin; aGvHD - acute Graft Versus Host Disease; cGvHD - chronic Graft Versus Host Disease

Table 2. Characteristics of factors influencing on the early chimerism status.

Transplant variables	Donor chimerism level (medium %)							
	+7 day	**p value**	**+ 14 day**	**p value**	**+ 21 day**	**p value**	**+ 28 day**	**p value**
Underlying diseases								
B ALL n= 31	58	p=0.9	100	p=0.3	100	p=0.79	100	p=0.58
T ALL n= 25	63		95		100		100	
Age of patient	58	p=0.98	95	p=0.66	100	p=0.73		p=0.86
< median (8.67 years) n=28	65		99		100		100	
=>median n=28							100	
Patient gender								
Male n=38	61	p=0.99	94	p=0.15	100	p=0.68	100	p=0.7
Female n=18	62		100		100		100	
Donor type								
Matched related n=25	49	p=0.06	90	**p=0.02**	100	_	100	_
Matched unrelated n=28	68		100		100	**p=0.01**	100	**p=0.02**
Mismatched related n=3	80		100		100		100	
Donor gender								
Male n=31	77	**p=0.003**	100	**p=0.041**	100	p=0.56	100	p=0.80
Female n=25	46		89		100		100	
Stem cell source								
Bone marrow n=47	60	p=0.64	96	p=0.20	100	p=0.32	100	p=0.19
Peripheral blood n=9	63		100		100		100	
Conditioning regimen								
Radiation-based n=41	60	p=0.33	96	p=0.40	100	p=0.40	100	p=0.43
Busulfan-based n=2	74		80		85		91	
Reduced toxicity n=13	67		100		100		100	
Serotherapy (ATG)								
YES n=28	49	**p=0.05**	94	p=0.11	100	**p=0.016**	100	**p=0.023**
NO n=28	68		100		100		100	
Number of CD34+ cells (median 4,47)								
< 4,47 x 10^6 n=35	58	p=0.41	94	**p=0.036**	100	p=0.08	100	p=0.19
> 4,47 x 10^6 n=21	67		100		100		100	

B ALL - B-cell acute lymphoblastic leukaemia; T ALL - T-cell acute lymphoblastic leukaemia; ATG - antithymocyte globulin

Figures

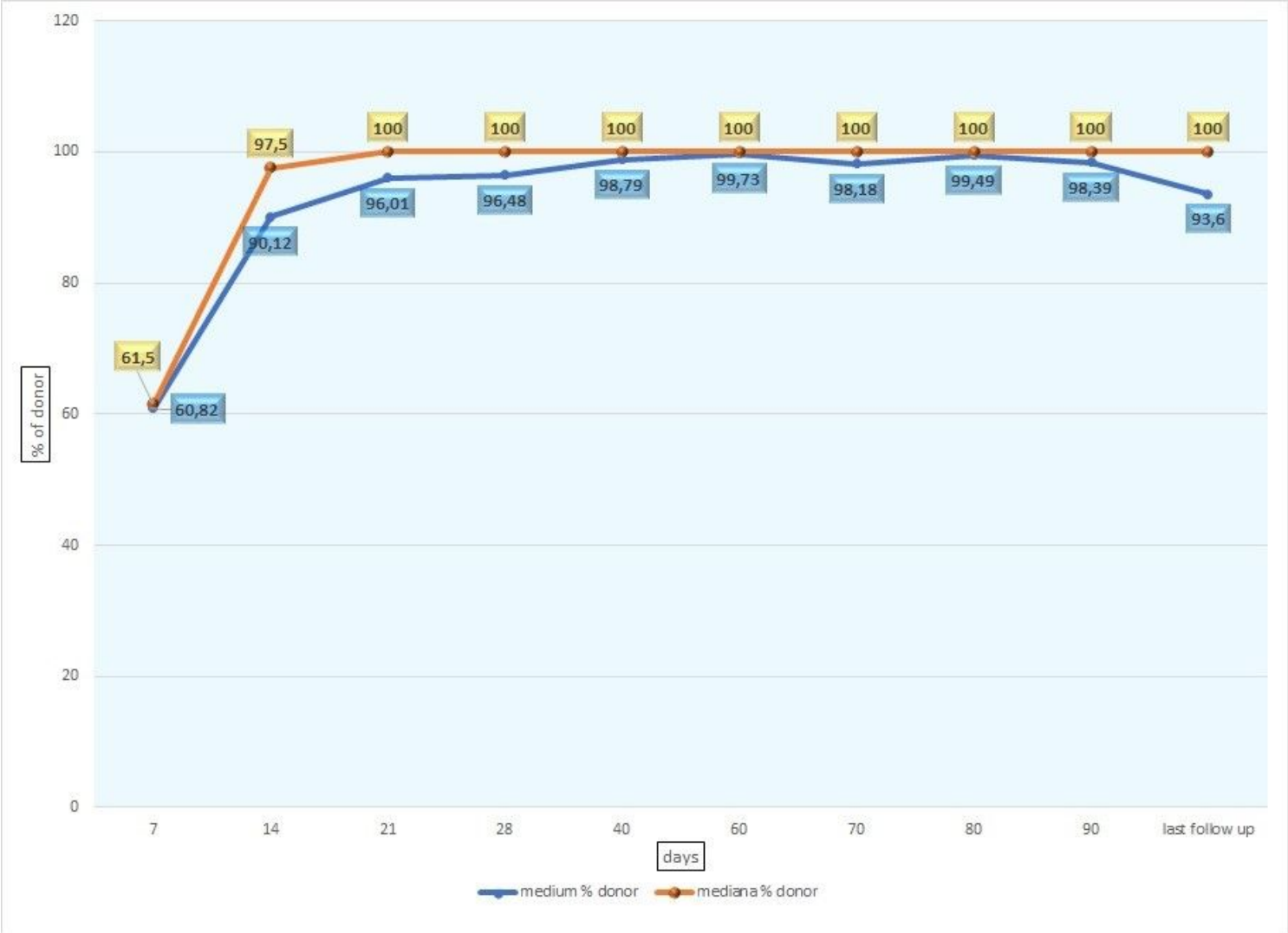

Figure 1

Figure 1. The kinetics of very early chimerism in the studied group of patients with ALL after allo-HSCT.

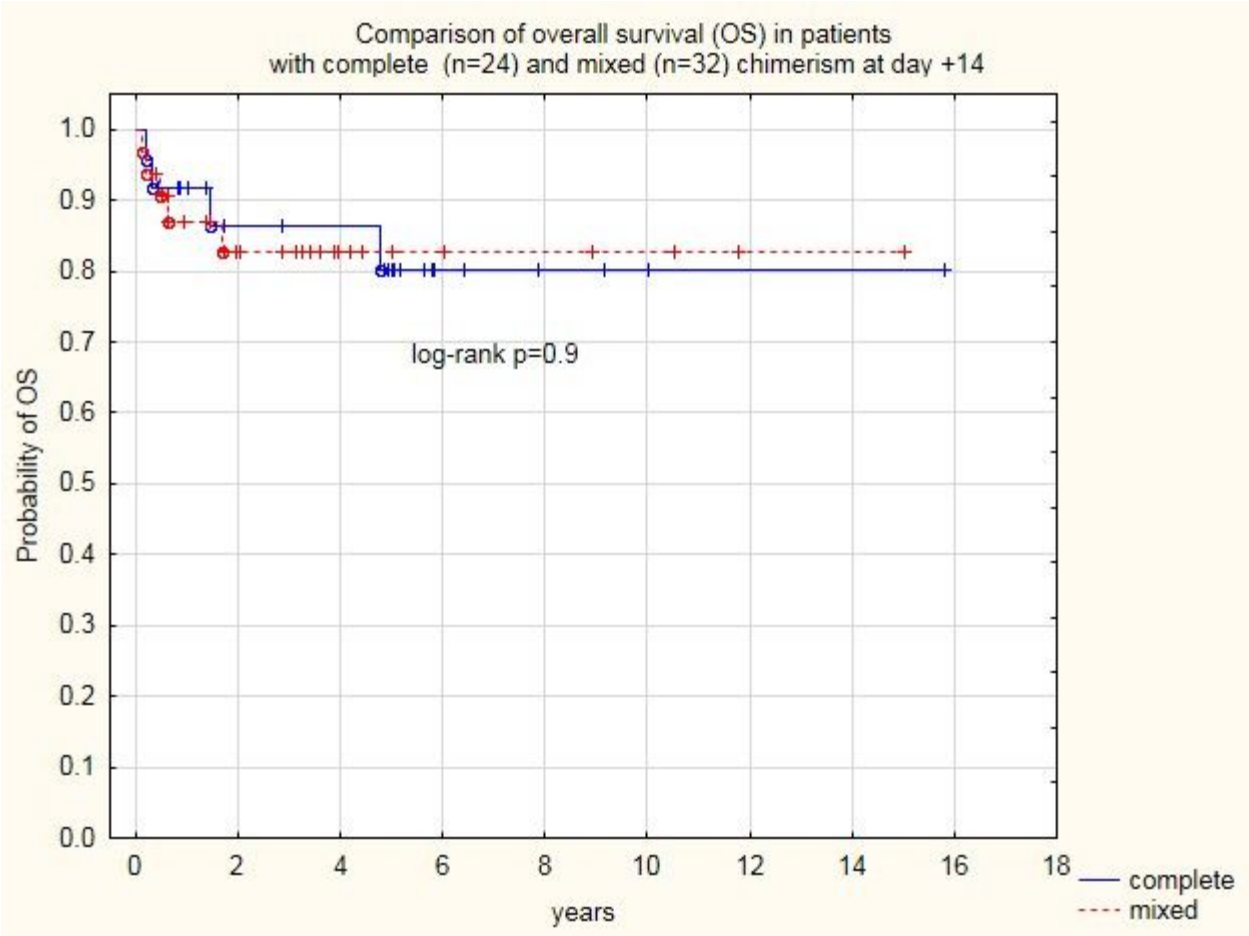

Figure 2

Figure 2. Comparison of overall survival (OS) in patients with complete donor chimerism and mixed chimerism at day +14.

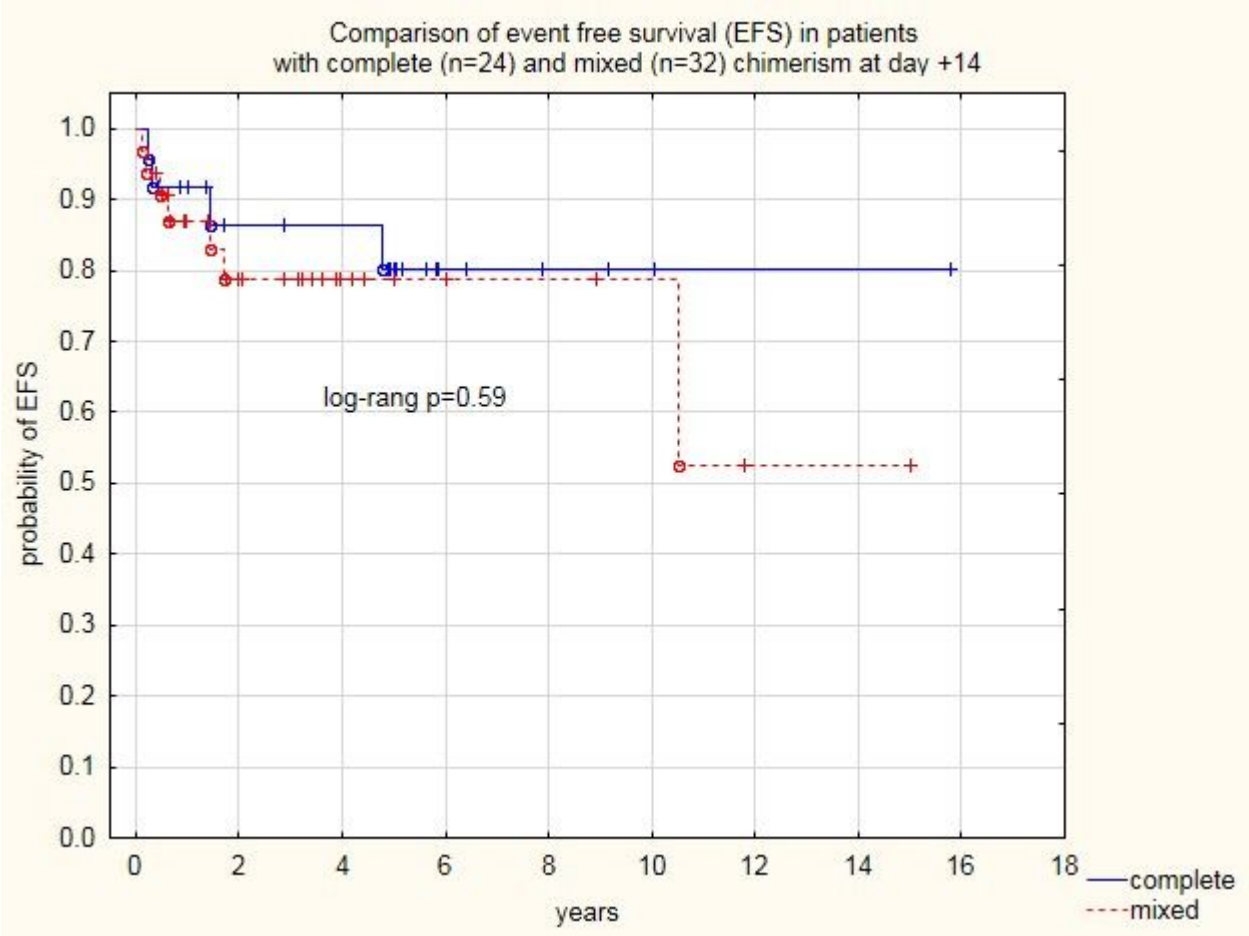

Figure 3

Figure 3. Comparison of event free survival (EFS) in patients with complete donor chimerism and mixed chimerism at day +14.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplement1.docx
- supplement2.docx