Influence of Mixed Chimerism on Outcome in Children With Anaemia After Haematopoietic Stem Cell Transplantation

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Abstract. *Background/Aim:* In patients with non-malignant diseases, mixed chimerism is not a rare phenomenon. The clinical impacts of chimerism following allogeneic haematopoietic stem cell transplantation (allo-HSCT) in children with congenital anaemia (CA) and severe aplastic anaemia (SAA) were analysed. *Patients and Methods:* We studied twenty-seven consecutive children with congenital and acquired anaemia who had undergone allogeneic haematopoietic stem cell transplantations. In the observed group of patients, the median of the follow-up was 6.12 years (2.00-14.8 years). *Results:* Overall survival (OS) did not depend on the type of disease $p=0.1$. OS did not significantly differ in patients who received more than $5 \times 10^6/\text{kg}$ stem cells (91%) and those who received less than $5 \times 10^6/\text{kg}$ (85%) ($p=0.61$). Two patterns of stable mixed chimerism (SMC) were observed: SMC (95-97% cells of the donor), and SMC with a fluctuation between 50-90% of the cells of the donor. None of the surviving patients received immunosuppression treatments of chronic Graft-versus-Host Disease (cGvHD). *Conclusion:* Our results showed that mixed chimerism did not influence the survival of children with congenital and aplastic anaemia following allo-HSCT.

Failure of bone marrow (BM) may occur as an isolated lack of one cell line or the complete absence of all three cell lines associated with pancytopenia, which is related to bone marrow

hypoplasia or aplasia. The most common congenital disorders of haematopoiesis include Fanconi anaemia (FA), Diamond-Blackfan anaemia (DBA), dyskeratosis congenita (DC), Shwachman-Diamond syndrome (SDS) and severe congenital neutropenia (SCN) (1). Acquired aplastic anaemia (AA) is a relatively rare disease of hematopoietic stem cells that is diagnosed in children. It is diagnosed after the exclusion of congenital marrow failure in patients between 6 and 9 years of age (2). A proportion of patients with AA may have an increased risk for conversion into myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) (3). Allogeneic haematopoietic stem cell transplantation (allo-HSCT) from a family donor is the first line treatment for severe AA as well as for congenital anaemia in children, but family donors are only available for 30% of patients with AA. In other cases, the intensive immunosuppressive treatment (IST) is applied. Bone marrow transplantations from unrelated donors or from mismatched family donors are used in the absence of effects from the first course of IST (upon evaluation of remission 120 days from the start of treatment) (4-6).

Haematopoiesis of the recipient and donor may coexist after transplantation and this state is called mixed chimerism (MC), which might result either in autologous recovery (AR) or transplant rejection. Haematopoiesis originating exclusively from donor cells is called complete chimerism (CC). The type of conditioning regimen, the source and number of transplanted cells, the type of donor/recipient, the graft prophylaxis, as well as the intensity of the post-transplant immunosuppressive treatment, can all have an influence on the evolution of haematopoietic chimerism (7, 8).

Following transplantation, four types of mixed chimeras are identified: i) a transient mixed chimerism (TMC), ii) a stable mixed chimerism (SMC) and iii) an increasing mixed donor chimera or iv) an “increasing mixed recipient chimera” (9). Clinical effects depend on the type of disease, the percentage of the donor’s cells and the type of mixed chimerism. In the case of non-malignant diseases, a therapeutic effect can be obtained even in a mixed chimerism (10).

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The aim of our study was to analyse the influence of a haematopoietic mixed chimerism following allogeneic haematopoietic stem cell transplantation on the survival of patients with congenital anaemias and acquired aplastic anaemia and to present the evolution of chimerism over time.

Patients and Methods

Twenty-seven consecutive children with congenital and acquired anaemia who had undergone allogeneic haematopoietic stem cell transplantations at the Department of Paediatric Haematology, Oncology and Transplantology of the Children's University Hospital in Lublin between 2003 and 2017 were included into the study.

SAA patients were qualified for transplantations from match-related donors as the first line of treatment based on the haematological criteria (a cellularity of BM of less than 25% and two of three of the following criteria being met: i) an absolute reticulocyte count $<20 \times 10^9/l$, ii) an absolute neutrophil count $<0.5 \times 10^9/l$ and iii) a platelet count $<20 \times 10^9/l$ without transfusion support. The remaining patients with SAA were transplanted after 1 or 2 cycles of immunosuppression treatment. Paroxysmal nocturnal haemoglobinuria was excluded in all patients. Patient characteristics are summarized in Table I.

Conditioning regimens were administered according to the current European Bone Marrow Transplantation (EBMT) recommendations (11). All but one patient received Anti-Thymocyte Globulin (ATG) rabbit as a part of the conditioning regimen in a total dose ranging from 5 to 7.5 mg/kg as the GvHD prophylaxis, at days -3, -2 and -1 before BMT. In addition, one boy transplanted from mismatched familial donor (MMFD) was treated with alemtuzumab, total dose 0.6 mg/kg at days -5, -4 and -3 before BMT.

Post-transplant chimerism was monitored from peripheral blood at the following time points: i) every week up to day +60, and ii) following every monthly sample collected up to 1-year post allo-HSCT. With regard to the status of the chimera, analysis of chimerism was performed every 2-6 months. When a patient presented a complete donor chimerism or a stable mixed chimerism, studies were performed every 6 months to 3 years following allo-HSCT. If there were any the clinical indications, the chimerism was analysed independently of the time points in the scheme of monitoring.

The genomic DNA isolated from mononuclear cells of peripheral blood constituted the investigation material. The procedure of isolating mononuclear cells was performed in a concentration gradient using Ficoll-Paque PLUS and in an aqueous solution of density 1.077 ± 0.001 g/ml (Amersham Biosciences, Inc., Piscataway, NJ, USA). Isolations of DNA were performed using the QIAamp DNA Blood Mini Kit (Qiagen, Germany). The quantity and quality of DNA were conducted using a spectrophotometer (BioPhotometr, Hamburg Eppendorf, Germany). It is recommended by the manufacturers to only use 1-2.5 ng DNA with the commercial AmpFISTR SGM Plus Kit (Applied Biosystem part of Thermo Fisher Scientific, Waltham, MA, USA). 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 1 minute, 59°C for 1 min and 72°C for 1 minute, with an additional elongation step for 45 minutes at 60°C (GeneAmp® PCR Systems 9700, Applied Biosystem part of Thermo Fisher Scientific). The following loci were amplified: D3S1358, vWA, D16S539, D2S1338 labelled with 5-FAM, Amelogenina X/Y, D8S1179, D21S11, D18S51 labelled

Table I. Characteristics of patients and transplantation method.

Characteristics	All patients
Diagnosis	
Congenital anaemia	
Diamond-Blackfan anaemia	6
Fanconi anaemia	4
Acquired	
Severe aplastic anaemia	17
Median age at transplant (range)	9.15 (0.7-17)
Gender	
Male	13
Female	14
Donor variable	
MSD	13
MUD	13
MMFD	1
Gender of donor	
Male	18
Female	9
IST treatment before HSCT	8
Conditioning regimen	
M	5
NM/RIC	22
Stem cells source	
BM	21
PB	6
Amount of cell CD34 (median)	
$<5 \times 10^6$	13
$>5 \times 10^6$	14

MSD: Matched sibling donor; MUD: matched unrelated donor; MMFD: mismatched familial donor; IST: intensive immunosuppressive treatment; M: myeloablative; NM/RIC: non-myeloablative/reduced; BM: bone marrow; PB: peripheral blood.

with JOE, D19S433, THO1 and FGA labelled with NED. Separation of the amplification products were made on an ABI PRISM 3130 automated DNA Genetic Analyzer (Applied Biosystem part of Thermo Fisher Scientific). The Gene Mapper 3.2 ID software (Applied Biosystem part of Thermo Fisher Scientific) was used to automatically determine the size of the amplified fragments (12).

The STATISTICA 13.0 software was used for all analyses. The Kaplan-Meier method and log-rank tests were used for the comparisons of survival analysis between groups. A value of $p < 0.05$ was considered statistically significant.

The study was approved by the Ethics Committee of the Medical University of Lublin (KE-0254/70/2010).

Results

In the observed group of patients, the median follow-up was 6.12 years (2.00-14.8 years). Overall survival (OS) was 87% (congenital-100%; SAA-78%; $p=0.1$) and did not depend on the type of disease (Figure 1A).

All surviving patients were transfusion-independent with normal PBC counts. Overall survival was 89% for myeloablative and 81% for non-myeloablative conditioning regimen ($p=0.75$)

Figure 1. Overall survival (OS) in AA patients. OS according to the disease (A), to the conditioning regimen (B), to the stem cells dose (C), and according to the type of chimerism (D).

(Figure 1B). OS did not significantly differ in patients who received more than $5 \times 10^6/\text{kg}$ stem cells (91%) and those patients who received less than $5 \times 10^6/\text{kg}$ (85%) ($p=0.61$) (Figure 1C).

Data for the transplant procedures, the chimerism status assessments at the analysed time points and the outcomes of the analysed patients are presented in Table II. The mixed chimerism was observed more frequently when the donor was a sibling ($r=0.4$, $p<0.05$). We did not observe correlations between the type of conditioning and the doses of stem cells and mixed chimerism. This study found no difference in the overall survival between patients with MC (90%) and CC (93%), $p=0.75$ (Figure 1D).

Complete donor chimerism was observed in 14/27 patients (seven patients with SAA, and seven patients with CA). In

our study, we observed two patterns of stable mixed chimerism. In the first pattern, the significant predominance of donor-derived cells (95%-97%) was observed in 3/27 patients. The second pattern showed a mixed chimerism with a fluctuation between 50%-90% of the donor cells in 7/27 patients (Table II). Stable mixed chimerism was observed in seven patients with SAA and two patients with CA, one of which was diagnosed with BDA and the other one with FA. Until that point they had not developed any malignant disease. Both patterns of mixed chimerism had no impact on the clinical outcomes. All patients with SMC were alive during the last follow-up and in good clinical condition.

One patient with DBA presented acute graft *versus* host disease (aGvHD) grade >1 and required treatment with

Table II. *Post-transplantation outcome according to chimerism status.*

Patient number	Type of disease	IST/ frontline HSCT	Conditioning regimen	Type of donor	Stem cell dose	Stem cell dose in mediana	aGvHD	Chimerism 10 day (% donor)	Chimerism 30 day (% donor)	Chimerism 90 day (%donor)	Chimerism 180 day (%donor)	Chimerism 365 day (%donor)	Last chimerism (%donor)	Type of chimerism status	Outcome
1	SAA	YES	NM	MUD	8.5	>5 mln/kg	NO	100	100	100	100	100	97	SMC	ALIVE
2	SAA	Frontline HSCT	NM	MSD	6.6	>5 mln/kg	NO	84	81	100	100	100	100	CC	ALIVE
3	SAA	Frontline HSCT	NM	MSD	2.5	<5 mln/kg	NO	100	100	100	100	100	100	CC	ALIVE
4	FA	ND	NM	MUD	1.8	<5 mln/kg	NO	100	100	100	100	100	100	CC	ALIVE
5	BDA	ND	M	MUD	4.44	<5 mln/kg	NO	100	100	100	100	100	100	CC	ALIVE
6	SAA	Frontline HSCT	NM	MSD	4.8	<5 mln/kg	NO	54	74	75	60	55	56	SMC	ALIVE
7	SAA	Frontline HSCT	M	MUD	6.6	>5 mln/kg	NO	84	100	100	100	91	94	SMC	ALIVE
8	SAA	Frontline HSCT	NM	MSD	3.5	<5 mln/kg	NO	55	80	80	65	61	60	SMC	ALIVE
9	SAA	Frontline HSCT	NM	MSD	1.32	<5 mln/kg	NO	100	100	65	84	53	69	SMC	ALIVE
10	FA	ND	NM	MUD	2.35	<5 mln/kg	NO	56	97	100	100	100	100	CC	ALIVE
11	FA	ND	NM	MUD	2.25	<5 mln/kg	NO	100	100	100	100	100	100	CC	ALIVE
12	SAA	YES	NM	MUD	6.5	>5 mln/kg	NO	ND	ND	ND	ND	ND	ND	ND	DIED
13	SAA	Frontline HSCT	NM	MSD	2.9	<5 mln/kg	NO	75	86	100	100	100	100	CC	ALIVE
14	SAA	Frontline HSCT	NM	MSD	8.8	>5 mln/kg	NO	43	88	69	71	73	72	SMC	ALIVE
15	SAA	YES	M	MUD	12.26	>5 mln/kg	NO	100	100	100	100	100	100	CC	ALIVE
16	SAA	YES	M	MUD	13	>5 mln/kg	NO	100	100	ND	ND	ND	100	CC	DIED
17	SAA	YES	M	MUD	14.7	>5 mln/kg	NO	100	100	100	100	100	100	CC	ALIVE
18	BDA	ND	M	MMFD	10.5	>5 mln/kg	NO	100	100	100	100	100	100	CC	ALIVE
19	BDA	ND	M	MUD	5.14	>5 mln/kg	NO	88	95	81	70	68	67	SMC	ALIVE
20	SAA	YES	NM	MSD	2.4	<5 mln/kg	NO	2	1	ND	ND	ND	ND	ND	DIED
21	BDA	ND	M	MSD	5.7	>5 mln/kg	NO	85	100	99	95	100	100	CC	ALIVE
22	SAA	Frontline HSCT	NM	MSD	14.7	>5 mln/kg	NO	100	100	86	66	72	87	SMC	ALIVE
23	BDA	ND	M	MUD	6.4	>5 mln/kg	YES	75	100	100	100	100	100	CC	ALIVE
24	SAA	Frontline HSCT	NM	MSD	2.16	<5 mln/kg	NO	22	100	100	100	100	100	CC	ALIVE
25	FA	ND	NM	MSD	2.1	<5 mln/kg	NO	35	56	80	55	80	90	SMC	ALIVE
26	SAA	YES	M	MUD	2.9	<5 mln/kg	NO	100	50	ND	ND	ND	ND	ND	DIED
27	BDA	ND	M	MSD	1.7	<5 mln/kg	NO	59	100	97	100	96	95	SMC	ALIVE

MSD: Matched sibling donor; MUD: matched unrelated donor; MMFD: mismatched familial; M: myeloablative; NM: nonmyeloablative; SAA: severe acquired anaemia; FA: Fanconi anaemia; BDA: Diamond-Blackfan anaemia.

steroids, with good clinical effects. In our group, we did not observe patients with chronic graft *versus* host diseases (cGvHD).

Four patients with SAA died. One patient's death was caused by post-transplant lymphoproliferative disease (PTLD) on day 18 following matched unrelated donor (MUD) HSCT. This patient was treated with two courses of immune-ablative therapy before HSCT. The second patient developed a fungal infection following matched sibling donor (MSD) HSCT. The third fatal outcome was caused by renal failure that rapidly progressed to multi-organ failure during the conditioning regimen, and the patient died on the second day following MUD HSCT. The fourth patient rejected the transplant and died due to appendicitis and complications following surgery.

Discussion

Recent data suggest that allogeneic haematopoietic cell transplantation is a well-established, sanative treatment for children with congenital diseases or acquired anaemia (13, 14). The increasing use of reduced-intensity regimens for non-malignant diseases causes an increasingly common appearance of mixed donor chimerism following HSCT (15). Therefore, monitoring the chimerism status is recommended following allogeneic SCT for congenital and acquired anaemia, and this monitoring should aim at detecting molecular engraftment and predicting the risks of early and late graft rejections (16, 17).

Quantitative fluorescence polymerase chain reaction (QF-PCR) method with fluorescent-labelled primers and the resolution of fragments *via* capillary electrophoresis used in our study is reliable, is standardized in the chimerism laboratory and is well described in the literature (18). This method remains the gold standard for the assessment of post-transplant chimerism (19).

Other published studies have shown that mixed chimerism may be sufficient for producing clinical effects in children with congenital and acquired anaemia. Svenberg *et al.*, have described the importance of chimerism in patients with non-malignant diseases following HSCT. The overall survival rate in this study was 87%, which claimed that despite the high incidence of MC, overall survival was promising (20). In our study, we retrospectively evaluated the association between the type of disease and the transplant outcome. According to a recent publication on HSCT in children with SAA, the outcome could be improved with the use of MUD BMT as up-front therapy, based on excellent results in patients below 20 years (21).

Similar to our results, the data published by Farraci *et al.*, have demonstrated that the 1- and 3-year overall survival rates were 87.4% and 80.5%, respectively, with a lower OS at 3 years in patients with CC compared to those with MC ($p=0.008$) (22).

In our patients, no correlation between overall survival and the conditioning regimen was observed. In a study performed on paediatric patients with immunodeficiency, transplantations using non-myeloablative regimens confirmed the efficacy of the procedure, even with a mixed chimerism. Despite this, the authors were concerned about the possibility of increasing infectious complications related to increased immunosuppression (23). Madden *et al.*, have described late follow-up beyond 2 years post-HCT in 43 children with non-malignant disorders who received transplantations using reduced intensity conditioning (RIC) regimens. Late graft rejections or disease recurrence was not observed during the long-term follow-up in these patients, despite the 33% incidence of MC (24).

In our study, no correlation was observed between overall survival and the stem cell dose. Park *et al.*, have performed a retrospective analysis of chimerism status and have observed that only the number of transplanted CD34⁺ cells had an influence on patients' chimerism status (23). This difference could be related to the fact that the analysis concerned a larger number of patients. They did not observe significant differences in survival between the CC and MC groups, similar to our results (23). Our study had some limitations, such as a small number of examined patients. The reason for the small number of patients was due to both the rareness of SAA in children and the fact that our study was a single centre study. Additionally, our short tandem repeat polymerase chain reaction (STR PCR) analysis was conducted on unsorted peripheral blood.

Hassan *et al.*, have demonstrated that 50% of their patients with SAA presented mixed chimerism (4-37% of the recipient cells), which was sufficient, and a growing tendency for graft rejection was not observed (25). Stikvoort *et al.*, have suggested that a long-term SMC is a rare phenomenon in aplasia following HSCT. Despite the limited number of patients, but with long-term observations, the analysis confirmed that a sibling donor was associated with stable MC development, similar to the result observed in our study (26). The same observation has been made by Stikvoort *et al.*, who has claimed that long-term mixed chimerism does not negatively affect the well-being or long-term outcomes of patients (27). The presence of donor-derived cells following HSCT is less important in patients with AA, because both cell lines can live together, due to the nature of the disease, which is non-malignant. It is different for patients transplanted due to CA, as persistence of recipient hematopoiesis increases the risk of malignant transformation (AML, MDS) (28). Therefore, the MC statement in these patients, should be an indication for more frequent monitoring of chimerism, also later during the post-HSCT period. In our cohort 2/10 patients with CA developed SMC, and in these patients chimerism was monitored every 2 months.

Lawler *et al.*, have investigated the chimeric statuses of 86 patients with SAA and FA via the STR-PCR method. Age, sex match, donor type, aetiology of aplasia, source of stem cells, number of cells engrafted, conditioning regimen, GvHD prophylaxis, occurrence of acute and chronic GvHD and survival were analysed. There was no correlation between donor age, sex-match, and number of cells engrafted or GvHD prophylaxis on chimerism status post-BMT. The study results indicated that patients with progressive mixed chimeras (PMC) were at a high risk of late graft rejection ($n=10$; $p<0.0001$). Monitoring of the chimeric status is a very important tool during cyclosporine withdrawal because it may facilitate therapeutic interventions and prevent late graft rejections in patients who have received transplants for SAA (29). In our study, the schedule of chimerism monitoring was more frequent, and, therefore, in one patient increasing recipient mixed chimerism was diagnosed early post-transplantation (on day +14). Despite cyclosporin discontinuation, the patient rejected the transplant and died.

A large amount of essential research into predicting acute and chronic GvHD has been done based on the early determination of post allo-HSCT haematopoietic chimerism. Lawler *et al.* have noted that there was an inverse correlation between the detection of recipient cells post-SCT and the occurrence of aGvHD ($p=0.008$) (29). Others have reported that acute and chronic GvHD are more common for patients with CC (22). Patients with mixed chimerism statuses following transplantation have been observed by Svenberg *et al.*, as having less acute GvHD (20). In our study, we observed only one patient with acute GvHD who presented with CC.

In our study, mixed chimerism (MC) did not influence the survival of children with congenital and aplastic anaemia following allo-HSCT. On the basis of the results, we suggest that mixed chimerism statuses (TMC and SMC) are safe for patients with AA, but in patients with CA persistence of recipient hematopoiesis may increase the risk of malignant transformation. For this reason, the therapeutic approaches (*e.g.*, the withdrawal of immunosuppressive therapy, the use of donor lymphocyte infusions or the administration of a second transplant) should be planned with consideration.

Our results emphasize that to obtain truly useful and worthwhile data concerning transplantation, we should use both serial chimerism tests and clinical observations.

Conflicts of Interest

There are no conflicts of interest regarding this study.

Authors' Contributions

ML, KD planned the study and were responsible for the study design. AZP, JZ, AM, MC, collected the clinical data. ML, AZP, KD, JK analysed and interpreted the data, wrote, and supervised the paper. All authors read and approved the final manuscript.

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References

- 1 Iwafuchi H: The histopathology of bone marrow failure in children. *J Clin Exp Hematop* 58(2): 68-86. 2018. PMID: 29998978. DOI: 10.3960/jslr.18018
- 2 Young NS, Calado RT and Scheinberg P: Current concepts in the pathophysiology and treatment of aplastic anemia. *Blood* 108: 2509-2519, 2006. PMID: 16778145. DOI: 10.1182/blood-2006-03-010777
- 3 Tripathi P, Tripathi AK, Kumar A, Ahmad R, Balapure AK, Vishwakarma AL and Singh RK: DNA aneuploidy study for early detection of chromosomal abnormality in patients with aplastic anemia: Prognostic and therapeutic implications. *In Vivo* 22: 837-844, 2008. PMID: 19181017.
- 4 Marsh JC: Treatment of acquired aplastic anemia. *Haematologica* 92: 2-5, 2007. PMID: 17229628. DOI: 10.3324/haematol.11107
- 5 Locassciulli A, Oneto R, Bacigalupo A, Socié G, Korthof E, Bekassy A, Schrezenmeier H, Passweg J and Führer M: Outcome of patients with acquired aplastic anemia given first bone marrow transplantation or immunosuppressive treatment in the last decade: a report from the European Group for Blood and Marrow Transplantation (EBMT). *Haematologica* 92: 11-18, 2007. PMID: 17229630. DOI: 10.3324/haematol.10075
- 6 Miano M and Dufour C: The diagnosis and treatment of aplastic anemia: a review. *Int J Hematol* 101: 527-535, 2015. PMID: 25837779. DOI: 10.1007/s12185-015-1787-z
- 7 Bader P, Niethammer D, Willasch A, Kreyenberg H and Klingebiel T: How and when should we monitor chimerism after allogeneic stem cell transplantation? *Bone Marrow Transplant* 35: 107-119, 2005. PMID: 15502849. DOI: 10.1038/sj.bmt.1704715
- 8 Lion T: Molecular monitoring after HSCT. Chimerism. *In: Apperley J, Carreras E, Gluckman E, Masszi T (eds) The EBMT Handbook* 16: pp. 281-287, 2012. Available from: https://ebmtonline.forumservice.net/media/16_2/tex/content_alt/EBMT_Handbook2012_CHAP16_2.pdf
- 9 Bader P: Documentation of engraftment and chimerism after HSCT. *In: Carreras E, Dufour C, Mohty M, Kröger N (eds) The EBMT Handbook*. Springer, Cham, Switzerland, pp. 143-147, 2019. DOI: 10.1007/978-3-030-02278-5_20
- 10 McCann SR, Crampe M, Molloy K and Lawler M: Hemopoietic chimerism following stem cell transplantation. *Transfus Apher Sci* 32(1): 55-61, 2005. DOI: 10.1016/j.transci.2004.10.006
- 11 Apperley J, Carreras E, Gluckman E, Gratwohl A and Masszi T: Principles of conditioning. *In: Apperley J, Carreras E, Gluckman E, Masszi T, editors. Haematopoietic Stem Cell Transplantation*. Genoa: Forum Service Editore; 128-144, 2008.
- 12 Lejman M, Drabko K, Styka B, Winnicka D, Babicz M, Jaszczuk I and Kowalczyk JR: Usefulness of post-transplant hematopoietic chimera monitoring by use of the quantitative fluorescence polymerase chain reaction method. *Transplant Proc* 49: 1903-1910, 2017. PMID: 28923646. DOI: 10.1016/j.transproceed.2017.04.013
- 13 Ringdén O, Remberger M, Svahn BM, Barkholt L, Mattson J, Aschan J, Le Blanc K, Gustafsson B, Hassan Z, Omazic B, Svenberg P, Solders G, von Döbeln U, Winiarski J, Ljungman P and Malm G: Allogeneic hematopoietic stem cell transplantation

- for inherited disorders: Experience in a single center. *Transplantation* 81: 718-725, 2006. PMID: 16534474. DOI: 10.1097/01.tp.0000181457.43146.36
- 14 Yoshida N, Kobayashi R, Yabe H, Kosaka Y, Yagasaki H, Watanabe K, Kudo K, Morimoto A, Ohga S, Muramatsu H, Takahashi Y, Kato K, Suzuki R, Ohara A and Kojima S: First-line treatment for severe aplastic anemia in children: bone marrow transplantation from a matched family donor *versus* immunosuppressive therapy. *Haematologica* 99(12): 1784-1791, 2014. PMID: 25193958. DOI: 10.3324/haematol.2014.109355
  - 15 Haines HL, Bleesing JJ, Davies SM, Hornung L, Jordan MB, Marsh RA and Filipovich AH: Outcomes of donor lymphocyte infusion for treatment of mixed donor chimerism after a reduced-intensity preparative regimen for pediatric patients with nonmalignant diseases. *Biol Blood Marrow Transplant* 21: 288-292, 2015. PMID: 25464116. DOI: 10.1016/j.bbmt.2014.10.010
  - 16 Dubovsky J, Daxberger H, Fritsch G, Peters C, Matthes S, Gardner H and Lion T: Kinetic of chimerism during the early post-transplant period in pediatric patients with malignant and non-malignant hematologic disorders: implication for timely detection of engraftment, graft failure and rejection. *Leukemia* 13: 2060-2069, 1999. PMID: 10602430. DOI: 10.1038/sj.leu.2401603
  - 17 Hoelle W, Beck JF, Dueckers G, Kreyenberg H, Lang P, Gruhn B, Fuhrer M, Niethammer D, Klingebiel T and Bader P: Clinical relevance of serial quantitative analysis of hematopoietic chimerism after allogeneic stem cell transplantation in children for severe aplastic anemia. *Bone Marrow Transplant* 33: 219-223, 2004. PMID: 14647253. DOI: 10.1038/sj.bmt.1704337
  - 18 Lion T, Watzinger F, Preuner S, Kreyenberg H, Tilanus M, de Weger R, van Loon J, de Vries L, Cave H, Acquaviva C, Lawler M, Crampe M, Serra A, Saglio B, Colnaghi F, Biondi A, van Dongen JJM, van der Burg M, Gonzalez M, Alcoceba M, Barbany G, Hermanson M, Roosnek E, Steward C, Harvey J, Frommlet F and Bader P: The EuroChimerism concept for a standardized approach to chimeras analysis after allogeneic stem cell transplantation. *Leukemia* 26: 1821-1828, 2012. PMID: 22395360. DOI: 10.1038/leu.2012.66
  - 19 Clark JR, Scott SD, Jack AL, Lee H, Mason J, Carter GI, Pearce L, Jackson T, Clouston H, Sproul A, Keen L, Molloy K, Najeem'deen Folarin, Whitby L, Snowden JA, Reilly JT and Barnett D: Monitoring of chimeras following allogeneic haematopoietic stem cell transplantation (HSCT): Technical recommendations for the use of Short Tandem Repeat (STR) based techniques, on behalf of the United Kingdom National External Quality Assessment Service for Leucocyte Immunophenotyping Chimerism Working Group. *Br J of Haematol* 168: 26-37, 2015. DOI: 10.1111/bjh.13073
  - 20 Svenberg P, Mattsson J, Ringden O and Uzunel M: Allogeneic hematopoietic SCT in patients with non-malignant diseases, and importance of chimerism. *Bone Marrow Transplant* 44: 757-763, 2009. PMID: 19421178. DOI: 10.1038/bmt.2009.82
  - 21 Dufour C, Veys P, Carraro E, Bhatnagar N, Pillon M, Gibson B, Vora AJ, Steward CG, Ewins AM, Hough RE, Fuente J, Velandi M, Amrolia PJ, Skinner R, Bacigalupo A, Risitanto AM, Socie G, Latour RP, Passweg J, Rovo A, Tichelli A, Schrezenmeier H, Hochsmann B, Bader P, Biezen A, Aljurf MD, Kulasekararaj A, Marsh JC and Samarasinghe S: Similar outcome of upfront-unrelated and matched sibling stem cell transplantation in idiopathic paediatric aplastic anaemia. A study on behalf of the UK Paediatric BMT Working Party, Paediatric Diseases Working Party and Severe Aplastic Anaemia Working Party of EBMT. *Br J of Haematol* 171: 585-594, 2015. PMID: 26223288. DOI: 10.1111/bjh.13614
  - 22 Faraci M, Bognasco F, Leoni M, Giardino S, Terranova P, Subissi L, Di Duca M, Di Martino D and Lanino E: Evaluation of chimerism dynamics after allogeneic hematopoietic stem cell transplantation in children with nonmalignant diseases. *Biol Blood Marrow Transplant* 24: 1088-1102, 2018. PMID: 26223288. DOI: 10.1111/bjh.13614
  - 23 Park M, Koh KN, Seo JJ and Im HJ: Clinical implications of chimerism after allogeneic hematopoietic stem cell transplantation in children with non-malignant diseases. *Korean J Hematol* 46: 258-264, 2011. DOI: 10.3346/jkms.2013.28.12.1723
  - 24 Madden LM, Hayashi RJ, Chan KW, Pulsipher MA, Douglas D, Hale GA, Chaudhury S, Haut P, Kasow KA, Gilman AL, Murray LM and Shenoy S: Long-term follow-up after reduced-intensity conditioning and stem cell transplantation for childhood nonmalignant disorders. *Biol Blood Marrow Transplant* 22: 1467-1472, 2016. PMID: 27164064. DOI: 10.1016/j.bbmt.2016.04.025
  - 25 Hassan R, Bonamino MH, Braggio E, Lobo AM, Seanez HN, Tabak DG and Zalcberg IR: A systematic approach to molecular quantitative determination of mixed chimerism following allogeneic bone marrow transplantation: an analysis of its applicability in a group of patients with severe aplastic anaemia. *Eur J Haematol* 73: 156-161, 2004. PMID: 15287911. DOI: 10.1111/j.1600-0609.2004.00296.x
  - 26 Stikvoort A, Gertow J, Sundin M, Remberger M, Mattsson J and Uhlin M: Chimerism patterns of long-term stable mixed chimeras posthematopoietic stem cell transplantation in patients with nonmalignant diseases: Follow-up of long-term stable mixed chimerism patients. *Biol Blood Marrow Transplant* 19: 838-844, 2013. PMID: 23462188. DOI: 10.1016/j.bbmt.2013.02.015
  - 27 Stikvoort A, Sundin M, Uzunel M, Gertow J, Sundberg B, Schaffer M, Mattsson J and Uhlin M: Long-term stable mixed chimerism after hematopoietic stem cell transplantation in patients with nonmalignant diseases, shall we be tolerant? *PLOS* 6: 1-19, 2016. PMID: 27152621. DOI: 10.1371/journal.pone.0154737
  - 28 Bonfim C, Ribeiro L, Nichele S, Bitencourt M, Loth G, Kolinski A, Funke VAM, Pilonetto DV, Pereira NF, Flowers MED, Velleuer E, Dietrich R, Fasth A, Torres-Pereira CC, Pedruzzi P, Eapen M and Pasquini R: Long-term survival, organ function, and malignancy after hematopoietic stem cell transplantation for fanconi anemia. *Biol Blood Marrow Transplant* 22(7): 1257-1263, 2016. PMID: 26976241. DOI: 10.1016/j.bbmt.2016.03.007
  - 29 Lawler M, McCann SR, Marsh JCW, Ljungman P, Hows J, Vandenberghe E, O'Riordan J, Locasciulli A, Socié G, Kelly A, Schrezenmeier H, Marin P, Tichelli A, Passweg JR, Dickenson A, Ryan J and Bacigalupo A: Serial chimerism analyses indicate that mixed haemopoietic chimerism influences the probability of graft rejection and disease recurrence following allogeneic stem cell transplantation (SCT) for severe aplastic anemia (SAA): indication for routine assessment of chimerism post SCT for SAA. *Br J Haematol* 144: 933-945, 2008. PMID: 19183198. DOI: 10.1111/j.1365-2141.2008.07533.x

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