



Chimerism in pediatric hematopoietic stem cell transplantation and its correlation with the clinical outcome



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ABSTRACT

Hematopoietic stem cell transplantation (HSCT) is the only hope to cure many inherited and acquired hematological disorders in children. Monitoring of chimerism helps to predict the post-transplantation events, with the intention to enhance the long-term disease free survival (DFS). The study aimed to investigate the importance of early chimerism detection to predict the clinical outcome following HSCT. The study included nine recipients (six β -thalassemia and three severe aplastic anemia patients) and their 10/10 HLA identical sibling donors. Chimerism detection was performed by analysis of short tandem repeat (STR) polymerase chain reaction (PCR) for detection and quantification of the relative amounts of donor and recipient cells present on day + 28. Peripheral blood (PB) was the main stem cell source for HSC transplantation. Disease free survival (DFS) was 71.4% while overall survival was 85.7% for PBSC transplants at the median follow up period of 4 years. The early detection of chimerism by PCR-STR analysis for children with β -thalassemia and aplastic anemia correlated with the outcome of HSCT in 8 (88.8%) patients. Complete chimerism was associated with disease-free survival while mixed chimerism and autologous patterns were associated with poor prognosis. In conclusion, early chimerism testing is clinically important in prediction of outcome after allogeneic HSC transplantation.

1. Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is currently the only approach for many non malignant hematological diseases in children [1]. In Egypt, the most common type of the hemoglobinopathies is β -thalassemia major (BTM), a disease usually associated with lower health related quality of life [2,3]. Since the prospect of gene therapy is limited to developed countries, allogeneic HSCT remains the only available definitive cure for children with this severe inherited disease in deficient resources. HSCT replaces the ineffective erythropoiesis associated with the disease with an allogeneic stem cell capable of effective erythropoiesis. Children suffering from transfusion dependent β -thalassemia, who underwent HSCT, experienced higher health-related quality of life than patients treated with transfusion and chelation [4].

Human leukocyte antigen (HLA) compatible stem cells from an unaffected identical sibling are regarded as the best strategy for β -thalassemia patients requiring allogeneic transplantation. Improving

the clinical outcome among patients receiving allogeneic HSCT is considered a challenge and progressively changes practice [5]. Bone marrow (BM) has been the preferred source of stem cells in β -thalassemia, however, a shift to peripheral blood stem cells (PBSC) has been suggested to prevent graft failure, for the potential of the graft-letting effect of the T-cell content of PBSC grafts. The PBSC transplants, despite providing faster engraftment and immune reconstitution, are associated with an increased incidence of graft versus host disease (GVHD), a serious complication of HSCT, and a lower 2-year survival than BM grafts [6].

There also has been an expanding increase in the use of peripheral blood (PB) as an alternative source of hematopoietic stem cells for severe aplastic anemia (SAA) especially in countries with limited resources [7]. In the Eastern Mediterranean region, the hemoglobinopathies accounted for 7% of HSCT while bone marrow failure syndromes comprised 12.2% of all HSCT performed [8]. SAA is an acquired and potentially fatal disease and HSCT from an HLA-matched sibling represents the initial treatment of choice for children with SAA.

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When treating patients at high risk of graft failure and infective complications, the utilization of PB is sometimes favored to promote early engraftment and independence from platelet and red cell transfusions. Nonetheless, a significantly inferior outcome was noted after PBSC transplantation, mainly due to the increased morbidity risk of GVHD as well as a lower 2-year survival [9].

The need for HSCT persists to rise, and out of necessity the improvement in the post transplant management continues. Surveillance of chimerism and the quantitative determination of donor-specific cells in the recipient have become critical for predicting the success of HSCT [10]. Complete donor derived hematopoiesis is crucial for maintenance of engraftment and prevention of recurrence of the underlying disease [11]. An accurate quantitative analysis of chimerism would allow early identification of patients with a high risk of graft versus host disease or those liable to disease relapse in addition to early differentiation between failure of engraftment and delay in engraftment [12].

2. Objective

The objective of our work was the early detection of chimerism after allogeneic HSCT in β -thalassemia and SAA in pediatric patients and the correlation of the results with the clinical outcome. Another purpose was to compare between short tandem repeat (STR) analysis and variable number tandem repeat (VNTR) analysis for detection of chimerism in the patients.

3. Materials and methods

This is a study of allogeneic HSCT for children with severe transfusion dependent β -thalassemia and severe aplastic anemia. The study included 6 β -thalassemia major class 2 and 3 SAA patients who underwent HSCT from their siblings at the Hematology and Bone marrow transplantation Unit in Cairo university Aboualreesh Monerrah pediatric hospital. The study was approved by the research ethics committee of Cairo University. Parental informed consents were obtained from the parents and assent forms from the donors and recipients were collected.

3.1. Patients and their respective donors

The donors of HSCT were HLA matched siblings. Patients with severe β -thalassemia were confirmed by hemoglobin electrophoresis, high-performance liquid chromatography, DNA testing and bone marrow examination. Their sibling donors underwent hemoglobin electrophoresis and bone marrow examination. For SAA patients and their HLA identical donors, chromosomal breakage studies were performed. Laboratory investigations included serum ferritin, screening for hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), cytomegalovirus (CMV), Epstein–Barr virus (EBV), and Toxoplasma. ABO and Rhesus (RH) blood grouping, and HLA typing were tested. HLA types were determined using polymerase chain reaction (PCR) followed by hybridization with a panel of biotinylated sequence-specific oligonucleotide probes (INNO-LiPA, Fujirebio). Chest X ray, abdominal and pelvic ultrasound, echocardiography and dental consultation were performed for all patients.

The patients were hospitalized 12 days before transplantation in β -thalassemia and 6 days in aplastic anemia. The patients received the conditioning regimen according to their diagnosis. In β -thalassemia, the protocol comprised buslfan 16 mg/kg/total dose on day -7 to day -4, antithymocyte globulin 11 mg/kg/day between days -5 and -1 and days +1 and +5, cyclophosphamide 30 mg/kg/day on day -5 to day -2, dexamethasone 0.2 mg/kg/day on day -12 to day 0, methylprednisolone 2 mg/kg/day between days -7 and +4 then the dose was reduced 50% every week, ciclosporin A 3 mg/kg/day on day -1 for 1 year after transplantation. In SAA, the preparative regimen was cyclophosphamide 50 mg/kg/day for 4 consecutive days on day -5 to day -2, fludarabine 120 mg/m² total dose on day -3 to day -1, dexamethasone

0.2 mg/kg/day between days -5 and 0, ciclosporin A 3 mg/kg/day on day -1 for 1 year after transplantation. Only one SAA patient (Patient 1) received total body irradiation (TBI) in a total dose of 10Gy on 5 fractions in 2 fields on day -9 to day -5.

3.2. Hematopoietic stem cell harvest from the donors

Five β -thalassemia patients and 2 SAA patients received peripheral blood stem cells (PBSC). One SAA patient received bone marrow stem cell transplant (BMT) while 1 β -thalassemia patient received umbilical cord blood (UCB) stem cell transplant. In PBSC transplantation, stem cell mobilization in the donors by granulocyte colony stimulating factor (G-CSF, 10 μ g/kg) subcutaneous injections for 3–5 days was followed by leukapheresis (COBE Spectra Apheresis System, Cobe Laboratories; Lakewood, CO). The cells were infused immediately after harvesting. For BMT, the donors received G-CSF (10 μ g/kg) for 3 days, then bone marrow was collected from the posterior iliac crests under general anesthesia and infused immediately after harvesting. In umbilical cord blood transplantation, 50 ml of fetal cord blood were collected immediately after the cord was cut, into a blood collection set containing citrate phosphate dextrose anticoagulant. The unit was cryopreserved and stored in cord blood bank, then thawed and infused on the day of transplantation. Red cells were depleted by centrifugation because of major ABO mismatch.

3.3. Estimation of CD 34 + stem cell dose

The concentration of CD34 + stem cells was measured based on four-parameter flow cytometry (CD45-PerCP/CD34-PE staining, side and forward angle light scatter) according to ISHAGE protocol by the International Society of Hematotherapy and Graft Engineering [13]. This allowed the verification of CD34 + cells as being dim for CD45 fluorescence and having low side scatter [14]. In the lymphocyte and monocyte gate, the CD34 + stem cell dose was calculated (CD34 + cells % \times lymphocytes % \times total white blood cell count \times total volume of the isolated mononuclear cells).

3.4. Detection of engraftment and clinical follow-up of the recipients

Major outcomes including engraftment and transplant-related complications such as infections, acute and chronic graft versus host disease (aGVHD, cGVHD, respectively), and death were recorded. Recipients received G-CSF (10 μ g/kg) daily subcutaneous injections on day +6 until the total WBC count reached \geq 1000 cells/cmm. The myeloid engraftment day was defined as the first of 3 consecutive days after transplantation in which the absolute neutrophil count was at least 500/cmm [15]. The patients received prophylaxis against infections with acyclovir 1500 mg/m²/day and fluconazole 6 mg/kg/day. CMV reactivation was defined as detection of the virus by PCR. All transplant recipients received prophylaxis against GVHD by ciclosporine A 3 mg/kg/day. Diagnosis and grading of GVHD was performed according to consensus criteria [16].

3.5. Detection of chimerism by short tandem repeat (STR)-fragment length analysis

3.5.1. DNA extraction

Peripheral blood samples (2 ml) were collected in sterile EDTA vacutainers from donors and recipients before HSCT and on day +28 from the recipients. DNA was extracted according to manufacturer instructions (High pure PCR Template preparation kit, Roche Diagnostics, Germany). The extracted DNA was stored at -80 °C until further analysis.

3.5.2. DNA genotyping of simple sequence-length polymorphic markers that encode for STR

STR multiplex assay was used to amplify 15 microsatellite loci and the Amelogenin gender-determining marker (AmpFlSTR® Identifier® PCR Amplification Kit, Applied Biosystems). PCR amplification of the polymorphic tetranucleotide STR markers was followed by electrophoresis (ABI Prism 3100-Avant Genetic Analyzer, Applied Biosystems) and analysis of the data (GeneMapper® ID-X 1.4 Software, Applied Biosystems). The fluorescence intensity pattern of each STR locus was translated into an electropherogram. Negative and positive controls were included.

3.5.3. Calculation of the percentage of donor and recipient DNA

To identify an informative locus, pretransplant samples were screened for STR markers for which recipient alleles had a different number of repeats than the donor alleles. Peak area ratios of donor and recipient alleles were formulated to express the chimerism levels [17]. The mean of measurements of the different markers were calculated for analysis. Quantitative determination of recipient and donor chimerism levels was used to estimate the degree of HSCT engraftment. Complete donor chimerism (CC) was considered if 95% or greater donor cells were detected in whole blood samples, while mixed chimerism (MC) was defined if donor chimerism was less than 95% [18]. Autologous recovery (AR) was reported if less than 5% donor cells were found.

3.6. Detection of chimeric status

Variable number tandem repeats (VNTR) were analyzed in all transplanted patients by a panel of 6 VNTR loci (Apo-B, D1S80, YNZ-22, 33.1, 33.4 and 33.6). Recipient and donor pre-transplant DNA and recipient post-transplant DNA was amplified. The resultant fragments were analyzed using gel electrophoresis [19].

3.7. Statistical analysis

Data were statistically described in terms of mean \pm standard deviation (\pm SD) or median and range for quantitative data. Qualitative data were presented as frequencies and percentages. Excel program was used for the data presentation.

4. Results

4.1. Clinical and laboratory data of the recipients pretransplant

The study included 6 children diagnosed as β -thalassemia major class 2 and 3 children with severe aplastic anemia, treated with allogeneic HSCT. Among the patients, there were 6 males (66.7%) and 3 females (33.3%) and their mean age was 6.4 ± 2.8 years (3–11.5 years) (Table 1). Only one patient was positive for HCV antibodies with undetectable viral load by PCR (Patient 4) while all the

patients were negative for HBV surface antigen and HIV 1 and 2. All the patients had positive serology for CMV IgG at baselinewith negative IgM, while EBV was negative in all patients. Three patients showed positive serology for Toxoplasma IgG with negative IgM. The mean ferritin level in β -thalassemia patients was 2740 ± 1680 ng/ml. BM examination showed normocellular to hypercellular BM with erythroid hyperplasia in β -thalassemia patients and markedly hypocellular BM in SAA patients. Chromosomal breakage analysis in SAA patients to exclude Fanconi's anemia revealed normal results.

The patients received HSCT from their 10/10 HLA identical siblings (HLA*A, B, C, DR, DQ). Six recipient donor pairs (66.6%) were ABO-mismatched and 3 pairs (33.3%) were sex-mismatched. The mean age of the donors was 6.0 ± 4.2 years. BM examination of all donors showed normal bone marrow while hemoglobin electrophoresis diagnosed 4 siblings of β -thalassemia patients with β -thalassemia trait. Seven (77.7%) patients received PBSC, one (11.1%) patient received BM transplant and one (11.1%) patient received UCB (Table 2). The median of peripheral blood CD34+ cells received by the patients was 15×10^6 cells/kg/dose.

4.2. Outcome and complications of HSCT

All patients successfully engrafted except one β -thalassemia patient who suffered primary graft failure. The median engraftment day was +17 in SAA and +19.5 in β -thalassemia. PBSC engraftment occurred within 8–23 days with only one occurring on day +47 (Table 2). The outcome of HSCT showed disease free survival (DFS) in 6 patients (66.6%) and graft failure with recurrence of the disease in 2 patients (22.2%). One (11.1%) patient suffered engraftment syndrome and subsequently died 49 days after transplant. For PBSC transplantation, the DFS was 71.4% and the overall survival was 85.7%, at the median follow up of 4 years. The use of cord blood as a source of stem cells was inferior as one patient with β thalassaemia showed primary graft failure. Infection was the most common complication of HSCT in 5 (55.5%) patients including CMV and Toxoplasma reactivation, and chest infection. Only one (11.1%) patient who received a PBSC transplant (Patient 8) developed acute GVHD of grade1, while none of the patients developed chronic GVHD.

4.3. Chimerism detection by STR analysis

Informative loci by PCR-STR analysis were demonstrated in all patients. The median number of informative alleles was 8 in the recipients (+Y in sex mismatched male recipient) and 9 in the donors (+Y in sex mismatched male donors). Moreover, the median number of informative loci was 9 (+Amelogenin in sex mismatch). Seven (77.7%) patients showed complete donor chimerism (96%–100% donor cells), one (11.1%) patient experienced mixed chimerism (90% donor cells) while one (11.1%) patient suffered autologous recovery (0% donor cells) (Fig. 1).

Table 1
Clinical data of HSCT recipients and donors.

Patient	Age (years)/sex Recipient	Age (years)/sex Donor	ABO/Rh group Recipient	ABO/Rh group Donor	Organo-megaly	Diagnosis of the patient	BTM Trait of Donor
1	8.5/M	4.5/M	A +	B +	No	SAA	ND
2	11.5/M	7/M	AB +	AB +	No	SAA	ND
3	4/F	12/F	A +	A +	HSM	BTM	Trait
4	6/M	12/M	A-	O-	HM	BTM	Normal
5	3/F	6/M	A +	A +	HSM	BTM	Trait
6	4/M	3/M	A +	O +	HSM	BTM	Normal
7	7/F	0/M	A +	AB +	No	BTM	Trait
8	9/M	2/M	O +	B +	SM	BTM	Trait
9	5/M	8/F	A-	O -	HM	SAA	ND

M: male, F: female, HSM: hepatosplenomegaly, HM: hepatomegaly, SM: splenomegaly, SAA: severe aplastic anemia BTM: β thalassemia major, ND: not done.

Table 2
Recipient HSCT transplantation data.

	Disease	Stem cell source	Stem cell (CD34 +)/kg/dose	Engraftment day	Infections	Complications	Outcome
1	SAA	PBSC	17.5 × 10 ⁶	+ 23	CMV reactivation Cerebral Toxoplasmosis	No	DFS d + 1489
2	SAA	BM	5 × 10 ^{8,a}	+ 17	No	No	DFS d + 1501
3	BTM	PBSC	3.5 × 10 ⁶	+ 8	Secondary infection	No	DFS d + 1670
4	BTM	PBSC	91 × 10 ⁶	+ 18	Hemorrhagic cystitis CMV reactivation Chest infection	No	DFS d + 1601
5	BTM	PBSC	16.7 × 10 ⁶	+ 47	CMV reactivation	Secondary graft failure	Relapse d + 120
6	BTM	PBSC	15 × 10 ⁶	+ 21	Chest infection	Engraftment syndrome	Died d + 49
7	BTM	UCB	3 × 10 ⁶	No	No	Primary graft failure Ablation therapy induced aplastic anemia aGVHD grade 1	Relapse d + 28
8	BTM	PBSC	5.6 × 10 ⁶	+ 22	No		DFS d + 895
9	SAA	PBSC	14 × 10 ⁶	+ 10	No	No	DFS d + 894

SAA: severe aplastic anemia BTM: β thalassemia major, PBSC: peripheral blood stem cells, BM: bone marrow, UCB: Umbilical cord blood, CMV: cytomegalovirus, aGVHD: acute graft versus host disease, DFS: disease free survival, d:day.

^a Nucleated cell dose.

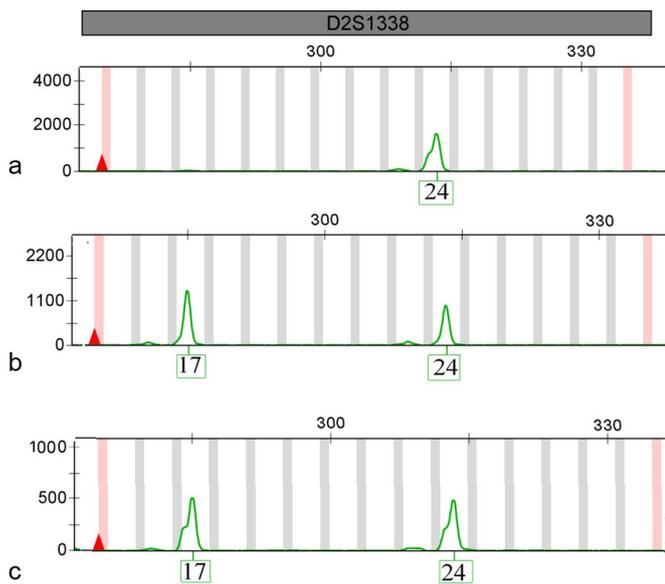


Fig. 1. A representation of chimerism by STR analysis in patient 3. Post transplant sample (c) on day 28, pretransplant recipient sample (a) and pretransplant donor sample (b).

The early assessment of donor chimerism by PCR-STR analysis at day + 28 correlated with the outcome of HSCT in 8 (88.8%) patients. Complete chimerism associated with disease free survival at the median follow up date of 4 years in 6 (66.6%) patients. Only one (11.1%) patient, who was a female β-thalassemia patient and received a PBSC transplant from her brother, achieved complete donor chimerism on day + 28 but suffered secondary graft failure on day + 120 related to CMV reactivation (Patient 5). One of the 6 patients experiencing complete donor chimerism developed acute GVHD. Mixed chimerism was associated with development of engraftment syndrome and subsequent death in one (11.1%) β-thalassemia patient who received a PBSC transplant (Patient 6). Autologous pattern on day + 28 in one (11.1%) patient was linked to primary graft failure after an UCB transplant (Patient 7).

Table 3
Comparison between STR and VNTR analysis of post transplant samples on day + 28 in patients.

Patient	VNTR results	STR results		Outcome
	Chimeric status	Chimeric status	Donor cells (%)	
1	No informative locus	CC	100	DFS
2	CC	CC	100	DFS
3	MC	CC	98	DFS
4	CC	CC	97	DFS
5	CC	CC	96	Relapse
6	CC	MC	90	Engraftment syndrome, Died
7	AR	AR	0	Relapse
8	MC	CC	97	DFS
9	CC	CC	96	DFS

VNTR: variable number tandem repeat, STR: short tandem repeat, MC: mixed chimerism, CC: complete chimerism, AR: autologous recovery, DFS: disease free survival.

4.4. Comparison of chimerism detection by STR and VNTR analysis

Donor chimerism was also examined by PCR-VNTR analysis. The results of STR and VNTR were in agreement in 5 (55.5%) patients (Table 3). One patient (11.1%) lacked an informative locus by VNTR analysis and showed complete donor chimerism by STR analysis. Discordant results were observed in 3 (33.3%) patients. In 2 of the 3 patients, PCR-STR analysis showed CC (97% and 98% donor cells) while VNTR analysis demonstrated MC. In one patient, MC (90% donor cells) by STR analysis was detected while VNTR showed CC.

5. Discussion

HSC transplantation from an HLA identical sibling is the treatment of choice for many hematological diseases in children. The probability of finding a compatible donor within the family in Egypt is 40% owing to large family size [20]. Peripheral blood was the main stem cell source in HSCT in the present study with disease free survival of 71.4% and overall survival of 85.7% at the median follow up period of 4 years. An early study conducted at the National Cancer Institute in Egypt

compared allogeneic PBSC and BM transplants as regards engraftment, incidence of GVHD and cost. Faster engraftment was observed in PBSC transplants, defined by neutrophil and platelet recovery and less days of neutropenic fever, leading to shorter hospital stay and less antibiotic and antifungal utilization. The incidence of acute GVHD in the PBSC transplant group was not higher supporting the hypothesis that GVHD severity is based on genetic disparities between donor and recipient [21].

On the other hand, in a large European Group for Blood and Marrow Transplantation registry-based study, PB grafts failed to reduce the incidence and mortality due to graft failure in severe aplastic anemia, but increased the risk of GVHD [9]. The study showed that BM should be the preferred stem cell source for matched sibling transplants, in patients of all age groups and particularly young patients. An adequate BM stem cell dose is anticipated to be associated with the improved outcome. However, in β -thalassemia the major reason for transplant failure is graft rejection due to previous multiple transfusions especially that most patients are referred relatively late in Egypt. With an allogeneic PBSC program and addition of antithymocyte globulin to the conditioning regimen to increase immunosuppression of recipients and overcome graft rejection, the overall and disease-free survival rates for β -thalassemia patients were 90% and 85%, respectively, at a median follow-up of 3 years [20].

Post-transplantation monitoring of chimerism facilitates the assessment of successful engraftment, however, it is also essential to predict outcome and impending complications such as graft rejection or GVHD. Chimerism testing by microsatellite analysis allows genotyping of STR markers which are highly polymorphic tetranucleotide repeat sequences interspersed throughout the genome [10]. One of the advantages of the STR-PCR test is the high rate of discrimination [22]. The PCR-STR analysis was informative in all our patients. Complete donor chimerism related to longer disease-free survival in the present study. Seven children showed CC on day +28 by STR analysis, of which six patients were disease free survivors at the median follow up period of 4 years and only one patient developed secondary graft failure at day +120 related to CMV reactivation. Although CC generally associates with less relapse and more frequent and severe GVHD, the incidence of acute and chronic GVHD in the pediatric population is low [23]. Indeed only one PBSC transplant patient suffered aGVHD of grade I and is a disease free survivor at the follow up date.

Mixed chimerism associates with shorter disease free survival and higher frequency of relapse. The identification of MC post HSCT, is to some extent, regarded as a phenomenon parallel to disease progression [24]. Our report is consistent with earlier observations particularly in β -thalassemia patients associating early mixed chimerism with inferior outcome [25]. In our patient group, one patient showed MC on day +28 by STR analysis, and developed engraftment syndrome on day +49. Engraftment syndrome refers to the development of fever without infection or skin rash or diarrhea within 24 h before granulocyte recovery. It is associated with an increased transplant-related mortality, from pulmonary and multi-organ failure [26]. In some cases, the engraftment syndrome may be a manifestation of a host-versus-graft (HVG) reaction and graft rejection. The intricate mechanisms of an alloresponse in either host-versus-graft or graft-versus-host involves activated T cellular interactions and pro-inflammatory cytokine production, upregulation of adhesion molecules and histocompatibility antigens, and tissue injury, contributing to the clinical picture of engraftment syndrome. Graft rejection may lead to a clinical presentation similar to a graft-versus-host reaction suggesting that competing HVG and GVH responses may be functioning in the setting of mixed lymphohematopoietic chimerism [26].

It was demonstrated that patients with increasing amounts of autologous patterns generally rejected their grafts subsequently [27]. The identification of a rise in the proportion of host cells in the early post-HSCT period strongly indicates a high liability of disease recurrence. In our study, one β -thalassemia patient showed autologous recovery on day

+28 by PCR-STR and experienced primary graft failure. Importantly, primary graft failure is the principal cause of treatment failure in patients with β -thalassemia [28]. However, the patient received an UCB transplant and less than adequate cell dose in UCB transplantation is a major factor associated with engraftment.

A comparative study between STR and VNTR analysis showed very good agreement and demonstrated that both markers are reliable tools for allogeneic engraftment analysis [29]. Our results of STR and VNTR-PCR were concordant in only 5 patients. Multiplex PCR based STR provides a high level of informativeness for detection of chimerism. This is further exemplified in Patient 1 where VNTR failed to demonstrate an informative allele. PCR-STR analysis in two patients showed 97% and 98% donor cells fulfilling the designation of complete chimerism while VNTR showed MC. The demonstration of the presence of mixed chimerism by qualitative methods is of minimal importance as regards the clinical consequences for the patient [12]. STR analysis is a semi-quantitative method where a percent of recipient cells are observed but may not be enough to fulfill the definition for MC [30]. Furthermore, STR analysis may be more sensitive than VNTR as one patient showed MC by STR analysis while the VNTR showed CC.

There are other known methods for chimerism detection such as fluorescent in situ hybridization (FISH). FISH analysis allows screening large number of cells and is a potent tool for monitoring engraftment with a high sensitivity and low false positive rates. The comparison of FISH analysis in patients transplanted from sex-mismatched donors showed an excellent correlation with the STR-PCR results [31]. However, STR analysis is not limited to sex mismatched HSCT and can be applied in any transplant setting.

The most common complication in our patients was infection (55.5%). Patients have a high incidence of infections following HSCT, due to the immunocompromised state associated with granulocytopenia and impairment of cell-mediated and humoral immunity [32]. CMV reactivation was the most common infection as all the patients were seropositive before transplantation. Close monitoring is crucial during the post-transplant period [20]. After allogeneic HSCT, around 30% of patients will experience reactivation of latent CMV infection, which is associated with 46% mortality [33].

We acknowledge limitations, principally the small size of the study. A larger sample size and more frequent samples may detect accurate correlation between the state of chimerism and the clinical outcome. Furthermore, aplastic anemia and β -thalassemia are heterogeneous conditions with a wide spectrum of clinical outcomes. Despite the limitations, the investigation of early donor chimerism is very important. Based on this case series, the early detection of chimerism for children with β -thalassemia and severe aplastic anemia is predictive for outcome of hematopoietic stem cell transplantation. Multiplex PCR based STR analysis is an accurate, semi-quantitative, highly informative method for detection of chimerism post allogeneic HSCT. Complete chimerism is associated with disease-free survival and mixed chimerism is associated with poor prognosis and necessitates close monitoring in order to begin the appropriate treatment.

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Declaration of interest

All authors have no conflict of interest.

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