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HEMATOPOIETIC STEM CELL TRANSPLANTATION MONITORING IN CHILDHOOD. HEMATOLOGICAL DISEASES IN SERBIA: STR-PCR TECHNIQUES

[1]ALEKSANDRA D. KRSTIĆ, [2]O. STOJKOVIĆ, [1,4]MARIJA GUĆ-ŠĆEKIĆ, [3,5]DRAGANA VUJIĆ, [3]DRAGANA JEVTIĆ, and [2]TANJA VARLJEN

[1]*Laboratory of Medical Genetics, Mother and Child Health Care Institute,* 11000 Belgrade, Serbia
[2]*Laboratory of Forensic Medicine, School of Medicine, University of Belgrade,* 11000 Belgrade, Serbia
[3]*Bone Marrow Transplantation Unit, Mother and Child Health Care Institute,* 11000 Belgrade, Serbia
[4]*Faculty of Biology, University of Belgrade,* 11000 Belgrade, Serbia

Abstract – Hematopoietic stem cell transplantation (HSCT) is a very successful method of treatment for children with different aquired or inborn diseases. The main goal of post-transplantation chimerism monitoring in HSCT is to predict negative events (such as disease relapse and graft rejection), in order to intervene with appropriate therapy and improve the probability of long-term DFS (disease free survival). In this context, by quantifying the relative amounts of donor and recipient cells present in the peripheral blood sample, it can be determined if engraftment has taken place at all, or if full or mixed chimerism exists. In a group of patients who underwent hematopoietic stem cell transplantation at the Mother and Child Health Care Institute, we decided to use standard human identfication tests based on multiplex PCR analyses of short tandem repeats (STRs), as they are highly informative, sensitive, and fast and therefore represent an optimal methodological approach to engraftment analysis.

Key words: Haematopoietic stem cells transplantation, chimerism, STR-PCR, hematological diseases

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INTRODUCTION

Allogeneic stem cell transplantation is an effective method of treating children with acquired and inborn hematological diseases. By quantifying the relative amounts of donor and recipient cells present, it can be determined whether engraftment has taken place, or if complete (CC) or mixed (MC) chimerism exists. Complete chimerism occurs when all of the bone marrow is from the donor, while mixed chimerism means that recipient cells are also present. In order to monitor chimerism after HSCT, different techniques can be used: conversion of blood group, karyotyping, XY-FISH analysis, and analysis of RFLPs, VNTRs, and microsatellites (STRs). We used the multiplex STR polymerase chain reaction as the most sensitive and sex-independent technique (C o m e a u et al., 2001; V e l i z a r o v a et al., 2005).

Short tandem repeats (STRs) are sections of highly repetitive DNA with tandem repeats of very short sequences (2-8 bp long). Tens of thousands of STR loci have been mapped in the human genome so far. The length and number of repeats within the STR vary considerably between individuals, which makes them highly informative for identity testing, genetic mapping, and forensic medicine. The availability of unique STR markers allows highly efficient differentiation of donor and recipient DNA in mixed samples. Fluorescent multiplex PCR provides the ability to analyze more than one marker at a time, increasing confidence in the relative quantification of donor and recipient cells in peripheral blood of the patient (B u n o et al., 2005; K h a n et al., 2004.

MATERIALS AND METHODS

We studied 10 patients after allogeneic sibling donor transplantation**.** Peripheral blood samples were taken for analyses from recipients and donors before transplantation, but only from recipients after engraftment. Isolation of DNA from these samples was performed by the salting out method, and the DNA diluted to less than 2 ng/μl by quantification of UV absorbance at 260 nm. For PCR, we

used the STR multiplex system for 16 markers (PowerPlex 16 Promega) following the manufacturer's guidelines, PCR being carried out in a Perkin Elmer thermocycler with the following protocol: 95°C for 15 min, 60°C for 1 min, and 70°C for 1,5 min through 10 cycles; then 90°C for 1 min, 60°C for 1 min, and 70°C for 1,5 min through 22 cycles; followed by 60°C for 30 min and 4°C soak. Products of PCR were analyzed on an ABI PRISM 310 genetic analyzer. Analyses were performed using GENESCAN and GENOTYPER software. Quantification of the two cell populations was calculated from the peak areas.

RESULTS AND DISCUSSION

In this work, we present our results after one year of following chimerism in a group of 10 patients after allogeneic sibling donor HSCT**.** Our aim was to establish the protocol for monitoring chimerism in pediatric patients, determine the optimal interval between two analyses, and compare the predictivness of mixed chimerism for relapse in different hematological diseases.

In our group of patients we monitored chimerism every 30 days in the first six months, then every 60 days to the end of one year and every 90 days during the second year after HSCT (Table 2).

Clinical characteristics of patients are shown in Table 1.

Patient #1, with anemia aplastica, had decreasing MC. Thirty days after HSCT, 90% chimerism was achieved, but it declined to 30% on day +196 after HSCT, as he evolved to MDS and died due to disease progression (Table 2).

Patients #2, #7, #10, with anemia aplastica, have achieved stable mixed chimerism so far. Figure 1 shows the example of MC in patient #2, with a sex-mismatched transplant on two informative loci: this patient is hetero-

Table 1. Patients characteristics. *AML – acute mieloid leukemia, NHL – non-Hodgkin lymphoma, HSCT –* haematopoietic stem cells transplantation*, CC – complete chimerism, MC – mixed chimerism PR - partial remission, TMA - thrombocitic microangiopathy associated with HSCT*

PATIENT	***DIAGNOSIS date***	***Age***	***Donor***	***CHIMERISM***	***OUTCOME***
#1	Anemia aplastica	1988.	mother	MC	exitus letalis 9 months after HSCT
#2	Anemia aplastica	1995.	sister	MC	alive 20 months after HSCT
#3	thalassemia major	1997.	brother	MC	graft rejection 12 months after HSCT
#4	AML relaps CNS	1987.	sister	CC	exitus letalis 3 months after HSCT
#5	AML	2003.	sister	CC	alive 13 months after HSCT
#6	AML	1985.	father	CC	alive 19 months after HSCT
#7	Anemia aplastica	1994.	sister	MC	alive 9 months after HSCT
#8	NHL PR II	1988.	brother	CC	exitus letalis 3 months after HSCT
#9	AML M7	2004	brother	CC	exitus letalis 4 months after HSCT
#10	Anemia aplastica	1990.	sister	MC	alive 7 months after HSCT

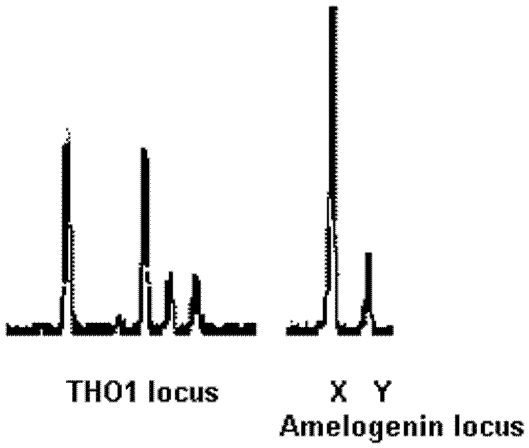

Fig. 1. Peak tracing for STR PCR analysis for THO1 and Amelogenin loci in patient #2 with MC.

zygous for both alleles of the THO1 locus, and the amelogenin locus is detected on both X and Y chromosomes.

Patient #3, with thalassemia major, retained stable MC until 196 days after HSCT; levels of donor chimerism then declined to 40% at 230 days after HSCT, when graft rejection occurred. He is now being prepared for new HSCT.

Patients #4, #5, #6, #9, with AML, achieved CC. Patient #4 had complications (*thrombotic microangiopathy associated with HSCT*) and died on day + 84 after HSCT. Patient #9 died 5 months after HSCT due to relapse. Patients #5 and #6 have survived for more than one year now. Figure 2 shows the example of patient #5 before and after HSCT.

Patient #8, with non-Hodgkin lymphoma, had CC for three months after HSCT, then died after disease progression. Decline in levels of donor chimerism was not observed (Table 2).

In two patients (#1, #3) with non-malignant disorders (anemia aplastica and thalassemia major), we observed MC and could predict a negative outcome, as decrease of donor chimerism was detected prior to graft rejection. Contrary to this, in one patient (#9) with AML and one patient (#8) with NHL, we observed CC, but death occured too quickly for us to intervene appropriately. We therefore conclude from our results that data obtained in STR-PCR analysis of chimerism correlate with clinico-hematological findings of remission or relapse of non-malignant diseases, and this is confirmed in the literature; however, our results (out of four patients with CC, two survived and two died) could not confirm the existence of a strong correlation between remission and hematopoetic CC, which is the most debatable issue in HSCT (A n t i n et al., 2001).

We conclude that STR analysis by fluorescent multiplex PCR allows simultaneous genotyping of 16 STR markers in a single reaction, which renders it highly informative and make possible analysis from a small amount of extracted DNA (< 2 ng/µl), since there is a limited sample source (because patients underwent strong chemotherapy before HSCT). Its high sensitivity (1-5%) and reproducibility makes this method extremely valuable for monitoring HSCT (F u n d i a et al., 2004; S e l l a t h a m b y et al., 2006). Although STRs are increasingly used for HSCT engraftment analysis, neither guidelines nor standards have been established (S c h i c h a m a n et al., 2002). Our recommendation for chimerism analysis is that it be performed at 1, 2, 3, 4, 6, 8, 10, and 12 months after transplantation, and then in the second year every 6 months or as needed, depending on the dis-

Table 2. The chronology of chimerism monitoring.

Patient	Days after BMT	Chimerism (% donor cells)
1.	32 63 155 196	90% 90% 60% 30%
2.	63 155 196 258,290	60% 70% 80% 70%
3.	73 119 151 196 230	50% 55% 60% 65% 42%
4.	26,57	100%
5.	92,124,160	100%
6.	27, 61, 90, 118,151	100%
7.	28, 62, 94, 119, 152	55% 66% 75% 72%
8.	27, 59	100%
9.	30, 62, 90	100%
10.	31, 60, 92, 121, 148	75% 66% 71%

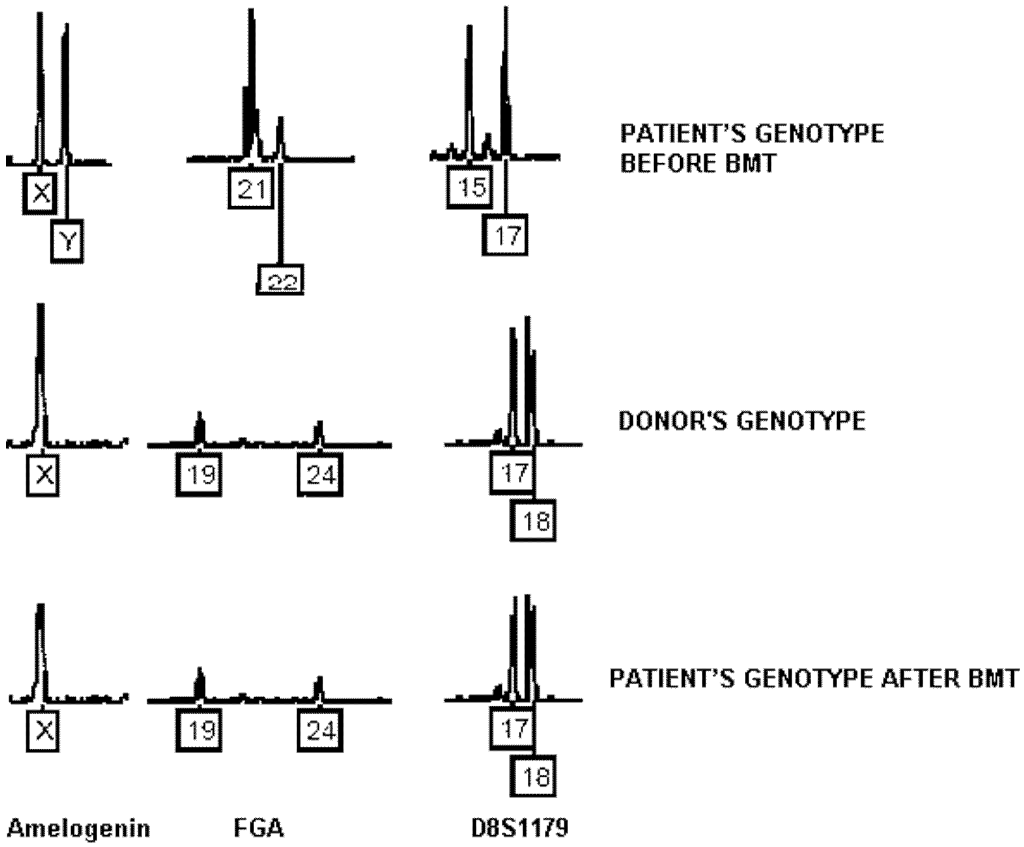

Fig. 2. Peak tracing for STR PCR analysis for Amelogenin, FGA and D8S1179 loci in patient #5 with CC.

ease and clinical findings. If the proportion of donor cells is observed to decline, ongoing monthly follow-up is warranted. Interventions to enhance donor engraftment must be considered on a disease-specific basis and ultimately by clinical rationale (A n t i n et al., 2001).

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УПОТРЕБА STR-PCR ТЕХНИКА ЗА ПРАЋЕЊЕ ХИМЕРИЗМА НАКОН ТРАНСПЛАНТАЦИЈЕ МАТИЧНИХ ЋЕЛИЈА КОД ДЕЦЕ У СРБИЈИ

[1]АЛЕКСАНДРА Д. КРСТИЋ, [2]О. СТОЈКОВИЋ, [1,4]МАРИЈА ГУЂ-ШЋЕКИЋ, [3,5]ДРАГАНА ВУЈИЋ, [3]ДРАГАНА ЈЕВТИЋ, [2]ТАЊА ВАРЉЕН

[1]Лабораторија за медицинску генетику, Институт за здравствену заштиту мајке и детета "Вукан Чупић", 11000 Београд, Србија
[2]Лабораторија за форензичку медицину, Медицински факултет, 11000 Београд, Србија
[3]Служба за трансплантацију костне сржи, Институт за здравствену заштиту мајке и детета "Вукан Чупић", 11000 Београд, Србија
[4]Биолошки факултет, Универзитет у Београду, 11000 Београд, Србија
[5]Медицински факултет, Универзитет у Београду, 11000 Београд, Србија

Трансплантација матичних ћелија хематопоезе је медицинска процедура у лечењу оболелих од урођених или стечених болести.

Значај праћења химеризма, након трансплантације матичним ћелијама хематопоезе је да се предвиде негативни исходи као што су релапс болести или одбацивање калема, како би могла да се примени адекватна терапија. Квантификацијом релативног броја ћелија даваоца и примаоца присутних у узорку периферне крви примаоца, могуће је одредити да ли је калем прихваћен, као и да ли је у питању комплетни или мешани химеризам.

У нашој групи од 10 трансплантираних пацијената користили смо реакцију ланчаног умножавања (PCR) кратких тандемских поновака (STRs), имајући у виду да поседују високу осетљивост и информативност, те стога представљају оптималну методу избора за праћење химеризма.