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Opinion-forming difficulties in disputed paternity cases resulting from bone marrow transplantation in biological father (a case report)

Summary

In the Department of Forensic Medicine and Medico-Legal Toxicology, Medical University of Silesia, Katowice, Poland before blood or buccal swab sampling for disputed paternity testing the parties are routinely asked whether they had bone marrow transplantation or not. The authors report the disputed paternity case in which the male defendant had hallogeneic bone marrow transplantation. In the tested trio, the child was six months old. For disputed paternity testing blood and buccal swab samples were taken from the defendant. As he had lost all his hair because of antineoplastic therapy, no hair follicles were taken. A female genetic profile (donor) was determined from the blood sample of the defendant, whereas the mixture of two profiles (donor and recipient) in the buccal swab. A genetic profile of the male defendant was finally distinguished from the mixture. In all tested loci the child had genetic markers which appeared also in the defendant. There was no paternity exclusion in any of the tested markers. Moreover, there was full genetic agreement between profiles of the child and the defendant. The report emphasizes the importance of the detailed history taking before biological material sampling for disputed paternity testing, which can prevent forming a false opinion.

Keywords disputed paternity cases, allogeneic bone marrow transplantation

Introduction

The number of allogeneic bone marrow transplantations is still rising. 297 such transplantations were performed in 2007, 397 in 2010 and 449 in 2012 respectively. The indication for allogeneic bone marrow transplantation can be: acute myeloid leukemia (AML), acute lymphoblast leukemia (ALL), myeolodysplastic syndrome (MDS), aplastic anemia (AA), chronic lymphatic leukemia (CLL), chronic myelocystic leukemia (CML), myeloproliferative syndrome (MPS), auto-immunological diseases, immune deficit or others [1,2]. Allogeneic bone marrow transplantation can result in chimerism [3,4].

The analysis of biological material from patients after bone marrow transplantation may be risky, as in the case of personal identification a false opinion can be formulated. In the Department of Forensic Medicine and Medico-Legal Toxicology, Medical University of Silesia, Katowice, Poland, before blood or buccal swab sampling for disputed paternity testing, the parties are routinely asked if they had blood transfused during the last three months and if they have ever had bone marrow transplanted. This procedure allows for taking suitable biological material (buccal swab, hair follicles, blood), using additional genetic markers for DNA investigation and finally for forming a yes-no opinion. Genetic material isolated from a buccal swab sample can lead to obtaining the mixture of STR alleles [5,6,7,8].

In this report the importance of a detailed history taking before biological material sampling for disputed paternity testing has been emphasized. In the described case alleles of 29 STR loci were determined in blood samples from the putative father and the child. There were no common alleles in 17 STR loci which, without the detailed history taking as well as homological gender in both the donor and the recipient, could result in forming a false opinion.

The genetic analysis of two biological materials (oral cavity epithelial cells, blood) allowed for confirming paternity with a higher probability than the one calculated for a buccal swab sample only. In the described case the bone marrow donor was a woman. In the case when a donor and a recipient are of the same gender, the risk of the error is higher.

Table 1

The results of the statistical analysis

Marker	Mother	Child	Defendant (buccal swab sample)	Formula (1) for PI value for defendant's genetic profile from buccal swab sample	PI value calculated according to the formula (1)	Defendant, blood sample	Allele of recipient (putative father)	Another allele of recipient (putative father)	Formula (2) for PI value for defendant's genetic profile from buccal swab and blood sample	PI value calculated according to the formula (2)	Allele frequencies
AMEL	XX	XY	XY	–	–	XX	–	–	–	–	–
CSF1PO	11/11	11/11	10/11/12	1/3p	1,23	10/12	11	10 lub 11 lub 12	2/3p	2,47	[10]
D1S1656	17.3/19.3	11/17.3	11/12/17/17.3	1/4p	2,86	12/17.3	11	17	1/2p	5,72	[13]
D2S441	10/11	10/10	10/11/14	1/3p	1,58	11/14	10	10 lub 11 lub 14	2/3p	3,17	[13]
D2S1338	20/23	19/20	17/18/19/26	1/4p	2,28	17/18	19	26	1/2p	4,56	[11]
D3S1358	16/17	17/17	15/16/17	1/3p	1,50	15/16	17	15 lub 16 lub 17	2/3p	3,00	[11]
D5S818	12/13	12/13	12/13	1/(p+q)	1,97	12/12	13	12 lub 13	1/(p+q)	1,97	[10]
D7S820	9/11	9/11	8/9/10/11	1/2(p+q)	1,43	8/10	9	11	1/(p+q)	2,86	[10]
D8S1179	13/15	15/15	10/13/15	1/3p	3,34	10/13	15	10 lub 13 lub 15	2/3p	6,69	[11]
D10S1248	14/15	14/15	14/16	1/2(p+q)	0,83	14/14	16	14 lub 16	1/4(p+q)	0,42	[13]
D12S391	18/18	18/18	15/18/21/22	1/4p	1,36	15/21	18	22	1/2p	2,71	[13]
D13S317	11/12	11/12	10/11/12	2/3(p+q)	1,11	10/11	12	10 lub 11 lub 12	5/6(p+q)	1,38	[10]
D16S539	12/13	11/12	11/12/14	1/3p	1,24	11/14	12	11 lub 12 lub 14	1/6p	0,62	[11]
D18S51	15/17	16/17	12/15/16	1/3p	1,50	12/15	16	12 lub 15 lub 16	2/3p	3,00	[11]
D19S433	14/14.2	14/15	13/15/16.2	1/3p	2,18	15/16.2	13	13 lub 15 lub 16.2	1/6p	1,09	[11]
D21S11	28/29	29/31	29/30/31	1/3p	3,72	29/31	30	29 lub 30 lub 31	1/6p	1,86	[11]
D22S1045	15/16	15/16	15/16/18	2/3(p+q)	0,99	15/18	16	15 lub 16 lub 18	5/6(p+q)	1,24	[13]
F13A01	6/7	5/6	4/5/6/7	1/4p	1,54	4/6	5	7	1/2p	3,08	[10]
F13B	8/10	10/10	8/10	1/2p	1,19	8/8	10	8 lub 10	3/4p	1,79	[10]
FESFPS	10/10	10/10	10/11/12	1/3p	1,28	10/11	12	10 lub 11 lub 12	1/6p	0,64	[10]
FGA	21/25	22/25	21/22	1/2p	2,74	21/22	21 lub 22	21 lub 22	1/2p	2,74	[11]
LPL	9/10	9/10	10/11	1/2(p+q)	1,13	11/11	10	10 lub 11	3/4(p+q)	1,70	[10]
Penta C	9/12	9/11	11/11	1/p	2,27	11/11	11	11	1/p	2,27	[14]
Penta D	9/13	13/13	9/13	1/2p	2,36	9/13	9 lub 13	9 lub 13	1/2p	2,36	[9]
Penta E	7/10	10/15	10/15/18	1/3p	4,50	10/15	18	10 lub 15 lub 18	1/6p	2,25	[9]
SE33	24.2/28.2	28.2/30.2	20/25.2/29.2/30.2	1/4p	5,10	25.2/29.2	20	30.2	1/2p	10,20	[13]
TH01	9.3/9.3	6/9.3	6/7/9.3	1/3p	1,29	9.3/9.3	6	7	1/2p	1,94	[11]
TPOX	8/11	10/11	8/10/11	1/3p	6,56	8/11	10	8 lub 10 lub 11	2/3p	13,12	[10]
vWA	16/17	17/17	16/17/18	1/3p	1,31	16/18	17	16 lub 17 lub 18	2/3p	2,62	[11]

Materials and methods

Total DNA was extracted from whole blood or buccal swabs using a DNA Genomic Mini AX or Sherlock AX kit (A&A Biotechnology, USA). Concentration of DNA was measured using a NanoDrop ND-1000 Spectrometer (ThermoFisher Scientific TK, Biotechnology, USA). PCR reactions were done according to the recommendations of the manufacturer of Power Plex ESX 17 kit (Promega, Germany), Power Plex 16 kit (Promega, Germany) and Power Plex CS7 kit (Promega, Germany). PCR products were separated and detected on the 3130 Genetic Analyzer (Applied Biosystems, USA). The alleles of the following STR loci were determined: AMEL, D3S1358, THO1, D21S11, D18S51, D10S1248, D1S1656, D2S1338, D16S539, D22S1045, VWA, D8S1179, FGA, D2S441, D12S391, D19S433, SE33, D5S818, D13S317, D7S820, CSF1PO, TPOX, PenTa D, Penta E, LPL, F13B, FES/FPS, F13AO1, Penta C. Additionally, alleles from chromosome Y were determined using a Yfiler test.

The genetic profile of the recipient (putative father) "was determined" with the reference to the genetic profile of the defendant determined from both his buccal swab and blood sample. PI values were calculated for both the genetic profile from the buccal swab and the genetic profile of the recipient. The frequency of a non-mother allele in the child was expressed by **p**. In the case when a non-mother allele was not possible to determine in the child, the frequencies of child's alleles were expressed by **p** and **q.** The allele frequencies were calculated using the formula: **(n + 1)/(2N + 1)** in which **n** = the number of a separate allele in the population sample, and **N** = the total number of the examined patients. The number of the alleles in population samples came from the literature data [9, 10, 11, 12, 13, 14]. In the case of genetic markers like D12S391 and VWA, a more informative one (D12S391) was used to calculate the total paternity index.

Results

As the defendant had had bone marrow transplanted two and a half years before his disputed paternity testing, his blood and buccal swab samples were taken for DNA polymorphism investigation. A female genetic profile (donor) was determined from the blood sample of the defendant whereas the mixture of two profiles (donor and recipient) from his buccal swab sample (Table 1 and Table 3). From the mixture, a genetic profile of the male defendant was finally distinguished. In all tested loci the child had genetic markers which appeared also in the defendant. There was no paternity exclusion in any of the tested markers.

In addition, paternity was confirmed by markers for chromosome Y which were the same in the defendant and the child (Table 2).

Table 2
The results of Y-STR polymorphism investigation

Loci STR	Child	Defendant (buccal swab sample)
DYS456	15	15
DYS3891	13	13
DY390	25	25
DYS38911	30	30
DYS458	17	17
DYS19	16	16
DYS385	12/14	12/14
DYS393	13	13
DYS391	11	11
DYS439	10	10
DYS635	24	24
DYS392	11	11
GATA H4	12	12
DYS437	14	14
DYS438	12	12
DYS448	20	20

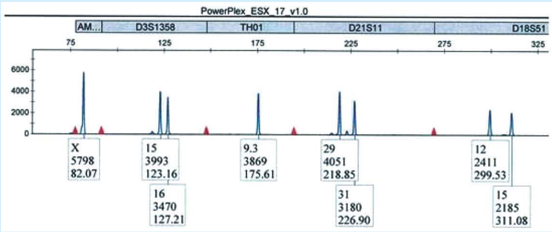
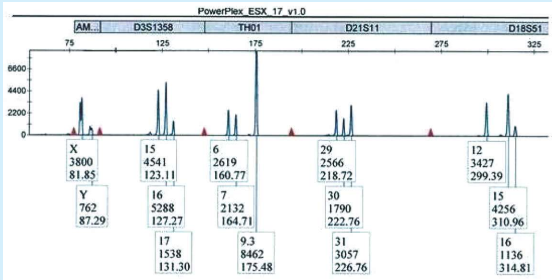
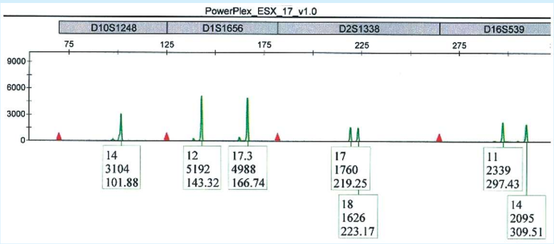
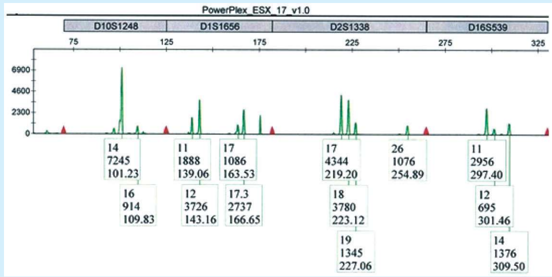
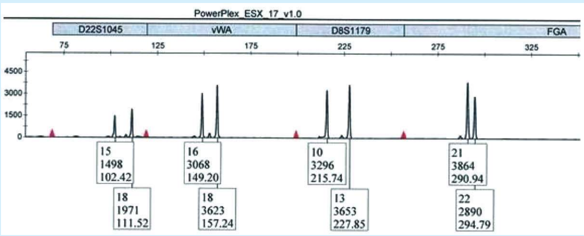
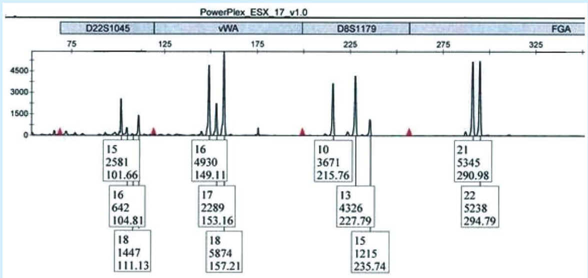
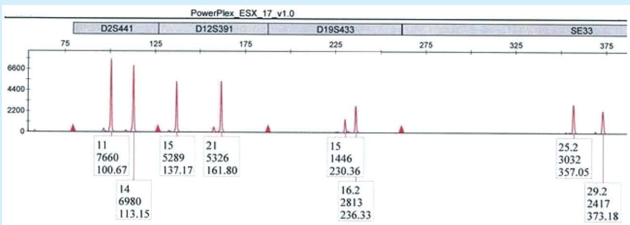
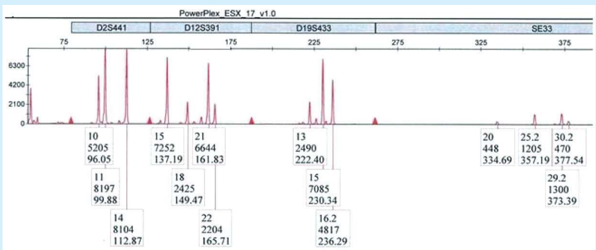
The obtained results have been shown in Table 1. For the genetic profile determined from buccal swab sample of the defendant, PI value was 2,52 x 107 (W = 99, 999996%). When the genetic profile determined from the blood of the defendant was taken into consideration, PI value was 8,50 x 10^2 (W = 99,99999999%). With reference to peak areas, the analysis of the DNA mixture from the buccal swab sample suggested that the alleles of the donor were a minor component (Table 3).

Discussion

In the described case the child was six months old and his biological father had had bone marrow transplanted two and a half years before his disputed paternity testing. These two facts meant that the child was conceived after bone marrow transplantation in the man. The analysis of DNA profiles in the child and the man confirmed paternity of the man.

The occurrence of blood – testis barrier results in specific autonomy of stem cells. This autonomy is characterized by a lack of inflowing stem cells from other niches in the body as well as a lack of differentiating these cells to spermatozoon. What is more, this hampers penetrating immune system cells which could cause damages to sex cells [16]. In Fig. 1 a seminiferous tubule is filled with cells from spermatozoon path which are protected by blood – testicle barrier (Fig. 1 Seminiferous tubule (cross section). M – myofibroblasts, P – sperm cells, S1 – primary spermatocytes, SP – spermatid, SA

Table 3
The comparison of genetic profiles of the recipient from his blood and buccal swab samples determined using a PowerPlex ESX17 kit

Recipient's blood sample (100% donor's chimerism)	Buccal swab sample (mixture of profile)
	
	
	
	

– spermatogonia type A, SB – spermatogonia type B, L – Leydig's cell, S – Sortoli's cells. Blood – testis barrier which is composed of: 1. continuous blood capillary epithelium, 2. myoid cells of lamina proporia and tight junctions between adjacent Sortoli's cells (Sortoli's cells are difficult to detect in routine hematoxylin and eosin staining).

One of the niches free of inflowing cells is the hair follicle. Most researches [5, 6, 17, 18], but not all [9] confirm the finding that the hair follicle does not contain the donor's genetic material regardless of the time after bone marrow transplantation. In the described case the man lost all his hair because of anti – neoplastic therapy, therefore no his hair follicles were taken.

In researches on chimerism, patients after bone marrow transplantation had hair follicles and blood and buccal swab samples taken.

The obtained results were in agreement with those in reports on cell chimerism in patients after bone marrow transplantation. They showed 100 % occurrence of the markers of the bone marrow donor in the blood sample of the recipient [5, 6]. As the time of the replacement is very quick, the analysis of a blood track in a crime scene can wrongly suggest third parties participation in a crime. The genetic profile in patients after bone

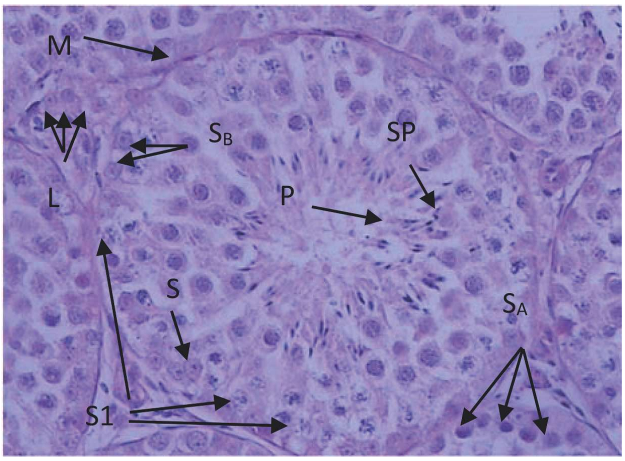


Fig. 1 Seminiferous tubule (cross section). M – myofibroblasts, P – sperm cells, S1 – primary spermatocytes, SP – spermatid, SA – spermatogonia type A, SB – spermatogonia type B, L – Leydig's cell, S – Sortoli's cells. Blood – testis barrier which is composed of: 1. continuous blood capillary epithelium, 2. myoid cells of lamina proporia and tight junctions between adjacent Sortoli's cells (Sortoli's cells are difficult to detect in routine hematoxylin and eosin staining)

marrow transplantation is very often the mixture of alleles, and a state of chimerism depends on the time after transplantation, steroid medicines or stomatitis.

Before buccal swab taking in patients after bone marrow transplantation, it is essential to evaluate their oral epithelium. Damaged oral epithelium can cause that DNA will be isolated from lymphocytes of peripheral blood. Thiede et. al. reported that the number of lymphocytes in saliva was 2-13 6000 cells/ml in patients without stomatitis and 1,1 x 10 ^{6} cells/ml [7] in patients with this symptom respectively.

Before buccal swab taking, a patient should rinse his oral cavity to remove inflowing and non – epithelial cells. It is important because of the fact that the donor's chimerism is higher in a saliva sample than in a buccal swab one [7, 20]. The examined man suffered from severe stomatitis.

Besides the right procedures of biological material sampling, a written acknowledgment or denial of the fact that bone marrow had been transplanted should be found in the sample protocol. It seems to be essential that unequivocal legal procedures concerning biological material sampling for disputed paternity testing should be established [21].

Conculions

In disputed paternity testing the knowledge on whether a patient has had bone marrow transplanted or not is of great importance.

Testing only a blood sample and no detailed history taking in the case when the donor and the recipient are of the same gender can give false results.

In the case of a patient after allogeneic bone marrow transplantation, a broad genetic profile from his buccal swab can prevent forming a wrong opinion.

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