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Artificial blood chimerism and graft-versus-host disease after liver transplantation

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Abstract. This study reports a case where early detection of graft-versus-host disease (GVHD) was possible by STR (short tandem repeat) analysis and demonstrates the value of this analysis for differential diagnosis. The aspects of artificial chimerism after solid organ transplantation for forensic investigations are pointed out.

Keywords: Chimerism; Graft-versus-host disease; GVHD; Liver transplantation

1. Introduction

After transplantation of solid organs a small amount of the donor's cells can be detected in the recipient's blood which usually indicates a good prognosis for organ survival in liver transplantation. In case of graft-versus-host disease (GVHD), however, donor's cells proliferate and produce an immune response against the recipient. In this case chimerism is observed to a higher extent. As a good concordance of HLA-antigens between donor and recipient represents a higher risk for GVHD, HLA-typing is carried out, but usually not considered for donor selection in liver transplantation.

Two months after transplantation of an ABO and Rh identical (O+) liver the recipient developed diarrhea and leucopenia, which were interpreted to be side effects of therapy with ganciclovir for cytomegalovirus infection. After cessation of ganciclovir, however, no improvement was observed and DNA-profiling was carried out on the recipient to exclude graft-versus-host disease as a possible reason for his condition. This study describes the importance of STR-typing in these cases.

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2. Materials and methods

A paraffin embedded bone marrow puncture taken in week 11 after liver transplantation, peripheral blood samples collected in weeks 16 and 18 (2 days before the patient deceased after multiorganic failure), buccal swabs (one of them slightly bloody) and eye brows of week 16 as well as paraffin sections from 21 different biopsies (prostata, trachea, heart, pelvic bone marrow, renal pelvis, colon, vertebral bone marrow, brain, both kidneys, aorta, both suprarenal glands, both lung lobes, liver, cardiac tissue, oesophagus, pancreas, stomach, and thyroid gland) taken during autopsy and a pre-transplantation blood sample of the donor were included in these investigations. DNA was extracted with the Qiamp DNA Mini Kit (Qiagen, Valencia, USA) or the Chelex method. Multiplex-STR-typing was carried out on the blood, the buccal swab and the hair samples of the recipient, the first bone marrow puncture and the donor's sample applying the AmpFlSTR®Identifiler™ PCR Amplification Kit (Applied Biosystems, Foster City, USA) according to the manufacturer's instructions. Singleplex-STR-typing of the highly polymorphic SE33 locus (fluorescein-labelled reverse primer) was performed on all samples [1]. The calculation of the percentages of the two-cell population was based on peak areas [2].

3. Results

A chimerism with a percentage of 80% donor and 20% recipient cells was observed in the first blood sample of the patient, with 10% donor's and 90% recipient's cells in the pure buccal swab sample and 70% donor's and 30% recipient's cells in the slightly bloody swab sample. Only the hair showed the recipient's DNA profile itself. The DNA profile of the blood sample taken 2 days before the patient's exitus was identical with the donor's profile; the patient's own DNA profile was no longer detectable (Table 1).

Table 1
Results of STR-typing

Sample origin	Donor	Recipient	Recipient	Recipient	Recipient	Recipient
Weeks after TX	0	11	16	16	16	18
Material	Blood	Bone marrow	Blood	Buccal	Hair	Blood
D8S1179	12, 13	12, 13, 14, 15	12, 13, 14, 15	12, 13, 14, 15	14, 15	12, 13
D21S11	28, 29	28, 29	28, 29	28, 29	28, 29	28, 29
D7S820	9, 9	9, 10	9, 10	9, 10	9, 10	9, 9
CSF1PO	10, 12	10, 11, 12	10, 11, 12	10, 11, 12	11, 12	10, 12
D3S1358	15, 17	15, 17, 18	15, 17, 18	15, 17, 18	17, 18	15, 17
TH01	6, 7	6, 7, 8, 9	6, 7, 8, 9	6, 7, 8, 9	8, 9	6, 7
D13S317	11, 13	11, 13	11, 13	11, 13	11, 13	11, 13
D16S539	11, 12	11, 12, 13	11, 12, 13	11, 12, 13	11, 13	11, 12
D2S1338	17, 23	17, 23, 26	17, 23, 26	17, 23, 26	17, 26	17, 23
D19S433	13, 13	13, 14	13, 14	13, 14	14, 14	13, 13
vWA	16, 17	16, 17	16, 17	16, 17	16, 16	16, 17
TPOX	8, 10	8, 9, 10	8, 9, 10	8, 9, 10	8, 9	8, 10
D18S51	14, 15	14, 15, 16	14, 15, 16	14, 15, 16	15, 16	14, 15
Amelogenin	X, Y	X, Y	X, Y	X, Y	X, Y	X, Y
D5S818	12, 12	11, 12	11, 12	11, 12	11, 12	12, 12
FGA	23, 26	21, 23, 25, 26	21, 23, 25, 26	21, 23, 25, 26	21, 25	23, 26
SE33	15, 20	15, 16, 20, 27.2	15, 16, 20, 27.2	15, 16, 20, 27.2	16, 27.2	15, 20

Table 2
Results of HLA-typing

	HLA-A	HLA-B	HLA-C	HLA-DR
Recipient	3, 24	7, 13	Cw6, w7	7, 15
Donor	3, 24	7	Cw7	7

To find out whether the chimerism could already have been observed in a paraffin embedded bone marrow puncture taken after the first onset of clinical symptoms, DNA profiling was carried out. 15% of the nucleated cells were shown to derive from the donor, while histopathology had just described hypocellular bone marrow without giving any clues to GVHD. Furthermore, chimerism was detectable in all of the 21 different post-mortem biopsies (ranging from 1% up to 62%), except the transplanted liver which only showed the donor's alleles. HLA-typing showed a high degree of histocompatibility between recipient and donor (Table 2) stimulating no immune response of the recipient against the HLA-antigens of the donor. As the recipient's HLA-B13, HLA-Cw6 and HLA-DR15 antigens were foreign to the donor, these incompatibilities represent a target for an immune response of the graft versus the recipient.

4. Discussion

In course of progression of clinical symptoms, the recipient's blood sample increasingly showed the donor's genotype and his DNA profile was found to be identical with the donor's profile at the zenith of graft-versus-host disease. Just his hairs were found to be free of the donor's DNA genotype and exhibited only his own alleles. Therefore, STR-typing of bone marrow samples should be performed whenever an early stage of graft-versus-host disease is suspected. It can be supposed that the chimerism would already have been detectable in the peripheral blood at this stage of the disease. At an advanced stage of GVHD caution has to be taken not to consider a single DNA profile as an exclusion of chimerism and therefore of GVHD as it might derive only from the donor. Hair samples of the recipient and material of the donor, if available, have to be investigated, in order to identify the two cell lines, as the major component does not necessarily represent the recipient's cell line. It is an important aspect for the forensic community that DNA profiles of liver-transplanted patients might be influenced or even replaced by the donor's profile, which was already shown in bone marrow transplanted patients [3,4] or other cases [5].

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