# The genetic profile of bone marrow transplanted patients in different vestiges of forensic interest

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## Background

**Hematopoietic stem cells** (HSC) present in the bone marrow are the precursors for all the blood cells.
In the **hematopoietic stem cell transplantation** (HSCT), cells of the lymphohematopoietic system of the patient are replaced by healthy HSCs of a donor. The main source of HSCs is the bone marrow (bone marrow transplantation). The HSCT is used to treat hematologic malignancies, but also non-hematologic malignancies and non-malignant disorders.

The coexistence of cells with different genetic origin (donor and receptor) after a HSCT is called **chimerism**. The quantitative study of chimerism after the transplant allows to know the success or failure of the graft, detect the existence of minimal residual disease, predict a possible relapse, etc., in order to apply the opportune therapy.

When a successful conditioning after the transplant is undergone, all of the HSCs and malignant cells of the patient are eliminated, which result in a complete replacement of donor cells (complete chimerism) in blood and bone marrow. However, due the potential of transdifferentiation of HSCs, it is demonstrated the coexistence of donor and receptor DNA (mixed chimerism) in several **non-hematologic tissues**. From a forensic perspective, biological vestiges of bone marrow transplanted patients may represent a challenge to legal-medical expertise, since the presence of chimerism can lead to errors in the interpretation of the genetic profile.

## Objectives

- **To analyse the genetic profile in biological vestiges of forensic interest (nail and skin epithelial cells) of bone marrow transplanted patients in order to establish the presence of chimerism.**
- To study the percentage of chimerism in nail and skin epithelial cells samples attending to the time elapsed from transplantation and other factors.
- To analyse the level of chimerism obtained in blood samples after HSCT when using 38 Insertion/Deletion (InDel) or 15 short tandem repeat polymorphisms (STR) for genotyping in order to compare their utility in the quantification of chimerism.

## Materials and methods

**1. Study samples**
- Peripheral blood
- Samples of nail and epithelial cells of epidermis (taken by adhesive tape or cotton swab) ceded by post-transplanted patients.
- Epidermis cells samples isolated from cutaneous biopsies of post-transplanted patients.

**2. DNA extraction**

**3. Amplification by multiplex PCR**
- Insertion/Deletion polymorphism (InDel)
- Short tandem repeat polymorphisms (STR)

**4. Sequencing**
- Capillary electrophoresis


**Figure 1.** Hypothetical loci of InDels and STRs. The InDels used as markers normally are biallelic, whereas STR can present many combinations depending on the length of the repeat unit and the number of repeated units.

**5. Data analysis**
- Profiles of samples of post-transplanted patients were compared with donor and receptor genetic profiles to obtain the level of chimerism by calculating the percentage of donor DNA in each sample. Calculations were made in the informative markers (loci that differ between donor and receptor) using the peak area of electropherograms.
- Statistical analysis: Pearson correlation coefficient (*r*) and Student's t-distribution. A $p < 0.05$ was established to interpret the existence of statistical significance.

## Results and discussion

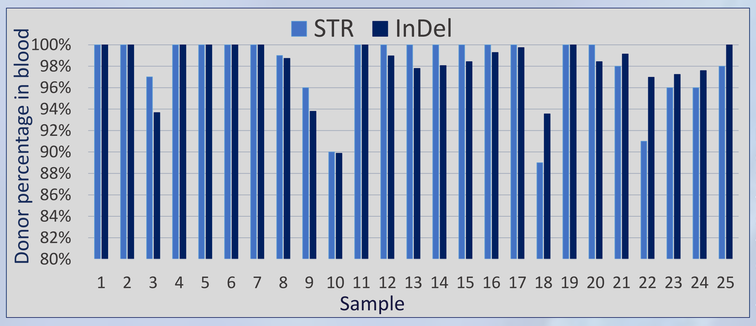


**Figure 2.** Percentage of donor DNA found in 25 blood samples of transplanted patients. Comparison between the donor percent obtained by using InDel or STR as technique.

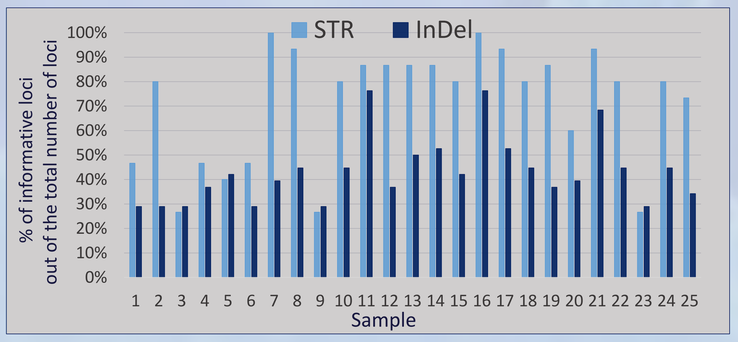


**Figure 3.** Percentage of informative loci in 25 blood samples of transplanted patients out of the total of available loci in each set (15 in the case of STRs and 38 for InDels).

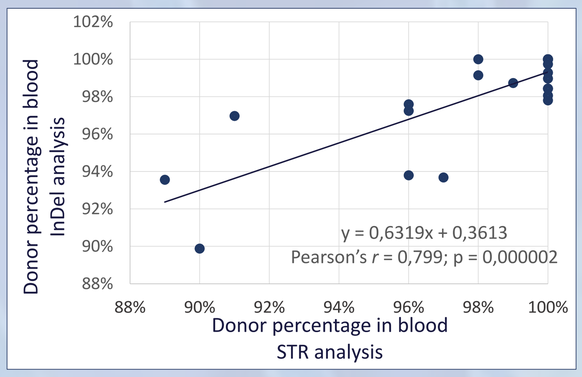


**Figure 4.** Comparison of the percentage of donor found in 25 blood samples of patients using the InDels and STRs analysis as technique.

There is a strong correlation statistically significative between the level of chimerism obtained with InDels and STRs analysis techniques.

The percentage of informative loci per patient is higher in STR than InDels, which was expected since InDels employed in this set are biallelic markers and the level of polymorphism of STRs is much higher.

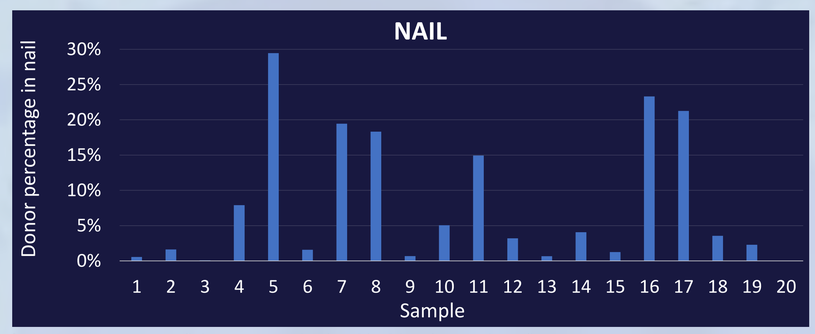


**Figure 5.** Percentage of donor DNA obtained in 20 nail samples of bone marrow transplanted patients.

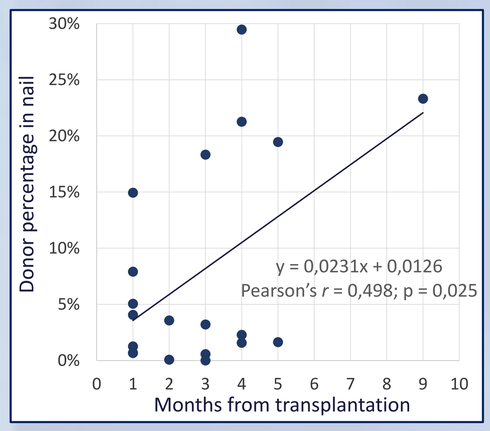


**Figure 6.** Percentage of donor DNA in 20 nail samples of bone marrow transplanted patients according to the time elapsed from transplantation.

Donor DNA is found in nail samples of bone marrow transplanted patients.

There is a positive correlation statistically significative between the percentage of chimerism found in nail and the time elapsed from the transplant. However, the nail chimerism does not correlate with the percentage of chimerism found in blood, age, type of conditioning prior to transplantation nor with graft-versus-host disease.

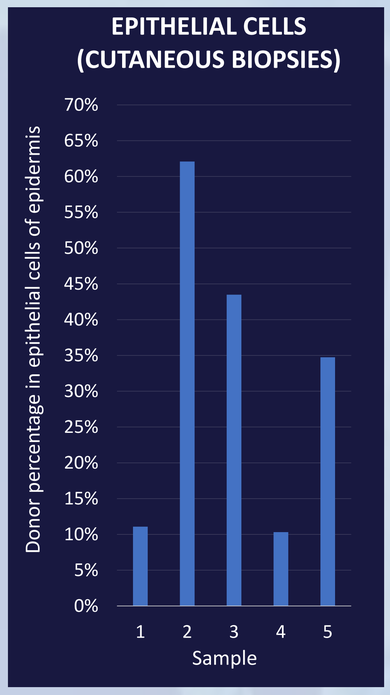


**Figure 8.** Donor DNA percentage obtained in 5 skin epithelial cell samples isolated from cutaneous biopsies of bone marrow transplanted patients.

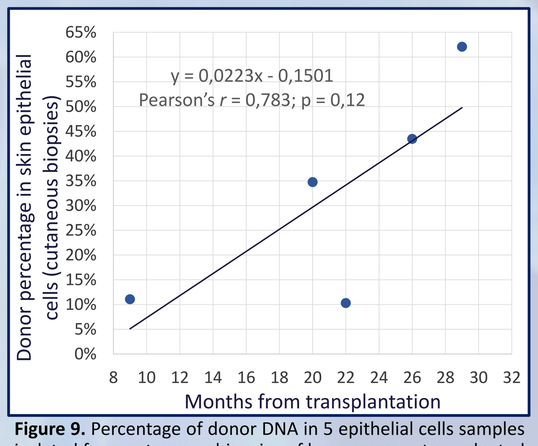


**Figure 9.** Percentage of donor DNA in 5 epithelial cells samples isolated from cutaneous biopsies of bone marrow transplanted patients according to the time elapsed from transplantation.

Donor DNA is found in epithelial cells of skin (epidermis) isolated from cutaneous biopsies of bone marrow transplanted patients.

There is a positive correlation between the percentage of chimerism in these samples and the time elapsed from transplantation, however it is not statistically significative. There is no relation between the chimerism in epidermis and the age or the level of chimerism found in blood.

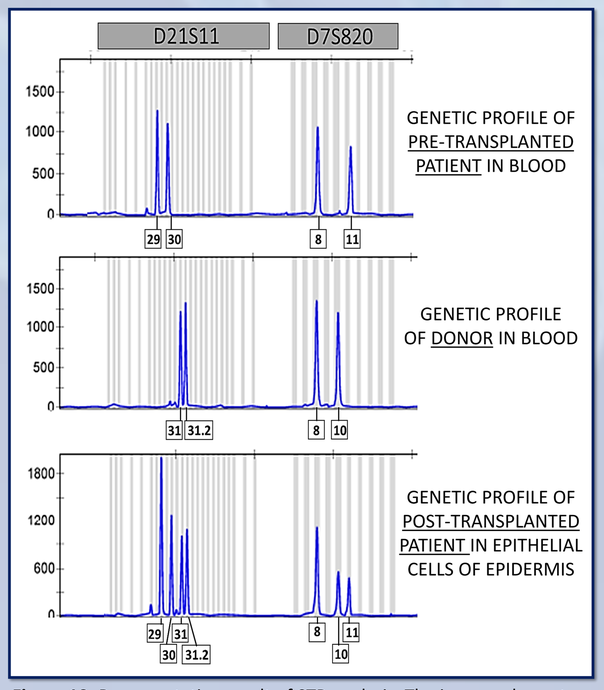


**Figure 10.** Representative result of STR analysis. The imagen shows two example markers of an electropherogram of epidermis epithelial cells sample of one bone marrow transplanted patient, as well as his corresponding donor and receptor profile for these markers. The post-transplanted sample presents chimerism.

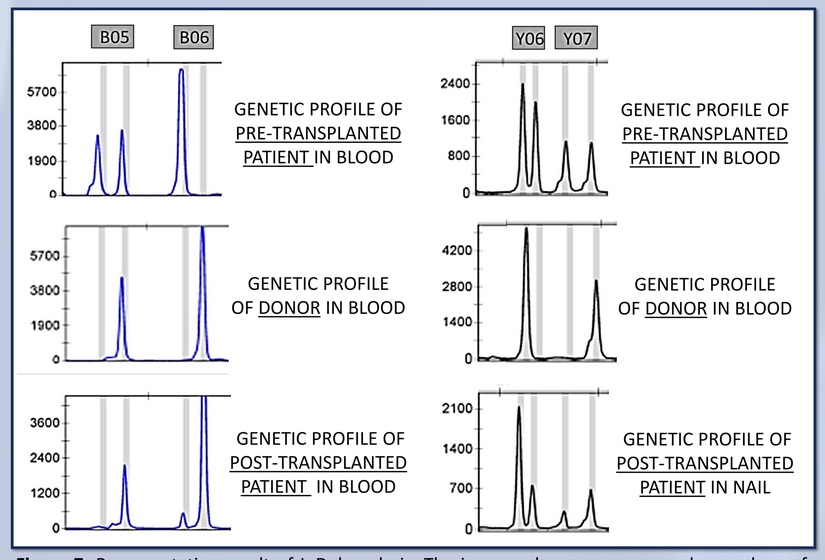


**Figure 7.** Representative result of InDel analysis. The imagen shows some examples markers of electropherograms of blood and nail samples of transplanted patients, and the corresponding donor and receptor profile for these markers. The post-transplanted samples present chimerism.

## Conclusions and future lines

**1-** The study of chimerism in blood and bone marrow samples of bone marrow transplanted patients should be carried out with efficient techniques in terms of the power of discrimination, cost and time. Although STRs are the most used method for this purpose, InDels allow to achieve very similar results and, in the case of partially degraded samples, where the STRs could not be amplified correctly, InDels would be very useful.

**2-** In the analysis of human identification markers of DNA extracted from nail and skin epithelial cells of bone marrow transplanted patients, a mixed human profile is obtained (the profile of the donor is amplified), which is a challenge from a forensic perspective.

**3-** The percentage of cells with donor profile in nail and epidermis increases when time elapses from the bone marrow transplantation. However, there is no correlation with the chimerism in blood, age, type of conditioning prior to the transplant or the presence of graft-versus-host disease, so they do not intervene in the differentiation of hematopoietic stem cells to these tissues.

As **future lines** it is proposed to: increase the sample size; optimize collection and DNA extraction techniques for those delicate supports which contain very few cell (such as adhesive tape or swab); try to find other factors which influence the level of chimerism; analyse the causes of the differences between the hematopoietic stem cells differentiation in hair and nail.

## References


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