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Chapter

Suitable Molecular Genetic Methods for the Monitoring of Cell Chimerism

Hana Cechova, Lucie Pavlatova, Monika Leontovycova and Milena Vrana

Abstract

The molecular analysis of individual hematopoietic chimerism at a defined time after allogeneic hematopoietic stem cell transplantation represents an important non-specific marker of posttransplant course. The monitoring of its dynamic allows the identification of patients at a high risk of relapse. A variety of methods are used for the monitoring of cell chimerism. It is necessary to use sensitive molecular genetic methods for early detection of the autologous hematopoiesis. Quantitative multiplex real-time polymerase chain reaction (PCR) analysis can serve as a very sensitive (0.01–0.1%), relatively quick, and inexpensive method to detect <1% of minor genotype. With an increasing ratio of minor genotype (>1%), it is more suitable to use short tandem repeats (STRs) for its analysis. Based on the differences in recipient/donor pair genotypes, at least two suitable informative polymorphisms located at different chromosomes can be selected. The combination of methods is appropriate, and the choice of the used method depends on the patient's actual chimerism status. The cohort of 207 patients monitored at the Institute of Hematology and Blood Transfusion was divided into three subgroups according to their chimerism status (complete chimerism (CC), microchimerism, mixed chimerism (MC)) 3 years after allogeneic hematopoietic stem cell transplantation (allo-HSCT). A significant difference in the 3-year survival and 3-year relapse rates in all three subgroups was found.

Keywords: chimerism, hematopoietic stem cell transplantation, real-time polymerase chain reaction, short tandem repeats

1. Introduction

The term chimerism comes from Greek mythology. A chimera is a creature with a lion's head, a goat's body, and a snake's tail. In biology, it is a unique state where cells from genetically different individuals coexist within one body [1].

A chimerism can spontaneously occur in several situations; probably the most common is the persistence of fetal progenitor cells in maternal blood [2]. On rare occasions, a chimerism can be developed in the uterus through the fusion of two genetically different zygotes (so-called tetragametic chimerism) or from a stem cell transfusion between dissimilar fraternal twins that share a placenta [3].

The latter possibility is an engraftment of maternal hematopoietic stem cells, especially in children with severe combined immunodeficiency.

Artificial cell chimerism can arise after transplantation of an organ, tissue, or hematopoietic stem cells (HSCT). The allogeneic HSCT (allo-HSCT) is one of the most used curative options for the treatment of hematological malignant and nonmalignant diseases, and for some diagnoses, it is currently the only available therapy. The long-term success of allo-HSCT depends on many factors such as an appropriate condition regimen (which destroys all leukemic cells), the state of patient in the time of HSCT (active disease vs. disease remission), the donor's age (T lymphocytes of younger donors are more willing to activate and destroy leukemic cells), the modification of the graft (T-cell-depleted graft vs. unmanipulated graft), and engraftment dynamic of HSC (it was proved that early achievement of full donor chimerism predicts lower relapse risk in acute lymphoblastic leukemia (ALL) patients) [4].

The analysis of cell chimerism is an integral part of the posttransplant monitoring of patients. In the immediate posttransplant period, this determination enables the identification of engraftment dynamics or graft failure, and it allows the early detection of a patient's increasing hematopoiesis which indicates a disease relapse.

The principle of the examination is based on the fact that each person has a unique DNA structure that comprises a set of highly variable polymorphisms; most polymorphisms are found in the noncoding regions of the genome [5, 6]. Thus, a comparison of the variable DNA polymorphisms of a patient and a donor provides information about the origin of the cells in almost every case.

2. Methods for the monitoring of cell chimerism

There are a number of molecular methods (e.g., sex-specific markers, fluorescence in situ hybridization (FISH), cytogenetic methods, erythrocyte phenotyping) that have been used for some time for the monitoring of cell chimerism; however, all of these techniques have their own limitations. They are laborious or time-consuming as well as have low informativity and sensitivity, (for a review, see [7, 8]).

2.1 History of molecular genetic methods

The first method of DNA analysis to take advantage of sequence polymorphisms was the method of restriction fragment length polymorphism (RFLP) where genomic DNA is digested with restriction endonucleases (restrictases) [9]. The size of the generated fragments is individually specific and depends on the various numbers of repetitive units in different individuals. All restriction fragments can be subsequently separated by gel electrophoresis. Southern blotting is used to transfer DNA from gel to filter membrane. The fragments are then detected by probe hybridization. For RFLP analyses it is necessary to extract high molecular weight undegraded genomic DNA.

The revolution in the monitoring cell of chimerism arose with the introduction of polymerase chain reaction (PCR) [10]. The first historical method based on PCR is amplified fragment length polymorphism (AFLP) [11]. Genomic DNA is digested by restriction enzymes, and the adaptors are subsequently ligated to the restriction fragments followed by selective PCR amplification with primers compatible to the adaptor's sequence. The amplicons are then separated by gel electrophoresis.

In laboratory practice, the most common current methods for long-term monitoring of cell chimerism are summarized in **Table 1**. They are based on the

	VNTR	STR	InDel	SNP
Type of polymorphisms	Length	Length	Insertion and deletion	Point
Sequence motif size	10–100 bp	2–6 bp	1–10,000 bp	1 bp
Analysis	PCR-specific repetitive sequence and subsequent to the fragment separation on agarose gel by electrophoresis	PCR-specific repetitive sequence and subsequent to the fragment separation on polymer by capillary electrophoresis	Quantitative real-time PCR	Quantitative real-time PCR
Sensitivity	1–5%	0.4–1%	0.01–0.1%	0.01–0.1%
Advantages	High informativity, unlimited quantitative determination	High informativity, unlimited quantitative determination	Highest sensitivity, rapid	Highest sensitivity, rapid
Disadvantages	Low sensitivity, time-consuming	Moderate sensitivity	Lower informative, lower accuracy in the majority of genotype quantification	Lower informative, lower accuracy in the majority of genotype quantification

VNTR, variable number of tandem repeats; STR, short tandem repeats; InDels, short insertions and deletions; SNP, single-nucleotide polymorphism; PCR, polymerase chain reaction.

Table 1.
Current possibilities of polymorphism analysis.

genotyping and quantification of different polymorphisms using PCR which provides an unlimited number of copies of a specific DNA segment based on a single copy.

2.2 Types of polymorphism

Length polymorphisms (loci) are repetitive tandem sequences and individual alleles (gene forms) that differ in the number of repeats of a sequence motif. These loci are widespread throughout the human genome and show sufficient variability among individuals in a population. They have become important in several fields including genetic mapping; linkage analysis; and human identity testing. These tandemly repeated regions of DNA are typically classified into several groups depending on the size of the repeat region such as variable number of tandem repeats (VNTRs) and short tandem repeats (STRs). The other types of polymorphisms are short insertions and deletions (InDels) or single-nucleotide polymorphisms (SNPs).

2.2.1 VNTR

VNTR polymorphisms are minisatellite DNAs with a total sequence length of up to 1000 base pairs and with a repetitive sequence motif of more than 6 base pairs. The benefit of VNTR analyses by PCR is a high degree of discrimination and unlimited quantitative determination, but, on the other hand, the disadvantage is the low sensitivity of the method (ranges from 1 to 5% depending on the analyzed VNTR locus and the combination of recipient-donor allele pair). The PCR products

(VNTR alleles) are visualized by fragment analysis via agarose gel electrophoresis, and when the donor's and the recipient's genotypes are detected in the sample together, the level of chimerism is determined densitometrically.

2.2.2 STR

STR polymorphisms are the repetitive sequences of microsatellite DNAs composed of up to one to six base pairs [12]. However, the most common STRs can reach, in their final range, from 100 to 500 base pairs [13]. The number of STR repetitive units may vary widely in populations. There are literally hundreds of STR systems which have been mapped throughout the human genome [14]. These STR loci are found on almost every chromosome. Since 1997, the National Institute of Standards and Technology (NIST) has compiled and maintained a Short Tandem Repeat DNA Internet Database [15] commonly referred to as STRBase. This STRBase includes used resources and the summary of human STR polymorphisms, their basic information, chromosomal location, overview of alleles, population studies, or available commercial kits. Observed alleles and annotated sequences for each STR locus are described along with a review of STR analysis technologies [16]. STR markers show a high level of polymorphism and consequently provide a significant degree of dissimilarity between individuals [17]. At present, they are considered to be the most informative genetic markers in the characterization of biological material. STR analysis is a very robust method with a sensitivity of 0.4–1% of minor genotype. Compared to VNTR, this method uses smaller quantities of DNA, including degraded DNA. STR polymorphisms may be amplified using a variety of PCR primers. Nowadays, a lot of multiplex kits are available on the market for human genotyping. The method is based on selective PCR of DNA segments with examined polymorphisms. PCR primers are fluorescently labeled. Separation of the fragments takes place subsequently in the genetic analyzer by capillary electrophoresis, as the carrier medium is used a polymer. In the genetic analyzer, fluorophores are excited by a laser ray to label DNA fragments. Fluorophores absorb laser light and emit light at a longer wavelength. Using an optical system, the emitted light is detected and recorded on a charge-coupled device (CCD) chip. STR alleles are visualized by specific software-like peaks. The area or height of peaks is used for quantification.

2.2.3 InDel and SNP

InDels are biallelic polymorphisms classified among small genetic variations, measuring from 1 to 10,000 base pairs in length [18, 19].

SNP is the smallest possible change in DNA sequence in individuals of a given population. SNPs are most often formed by a point mutation mechanism that is substitution-like, less often by deleting or inserting at a particular DNA site. The distribution of SNPs in the genome is not homogeneous. More often, they occur in noncoding regions, on average 1 of 1000 bp in any selected region of the genome.

SNP and InDel analysis are performed by real-time quantitative polymerase chain reaction (RQ-PCR) with specific primers designed to contain appropriate insertions or deletions or point-to-point substitutions. Real-time PCR allows accurate quantification of amplified products by detection of fluorescence released during the exponential phase of the reaction. This method can use the non-specific intercalating fluorescent DNA-binding dye SYBR Green that binds all double-stranded DNA [20]. More often TaqMan technology is used [21], which utilizes a fluorescent-labeled target-specific probe resulting in an increased specificity and

sensitivity compared to the SYBR Green method. Additionally, a variety of fluorescent dyes are available so that multiplex PCR can be used to simultaneously amplify and detect many sequences. The TaqMan probe consists of a fluorophore covalently attached to the 5′ end of the oligonucleotide and the quencher at the 3′ end. If the probe is in an intact state, even when the probe is hybridized to the target sequence, the emitted fluorescence is suppressed by the quencher. Only during the elongation phase, when the Taq polymerase with its 5′ exonuclease activity degrades the specifically bound TaqMan probe complex and releases the fluorophore from the quencher, can fluorescence occur and then be measured. The fluorescence intensity is directly proportional to the amount of PCR product. The calculation of the relative amount of target DNA in a sample is done by comparing the Ct values (the threshold cycle, at which the emitted fluorescent signal exceeds the statistical significance level). The resulting portion of the gene of interest (GOI) is calculated by the ΔΔCT method [22]. Data are normalized with the reference (housekeeping) gene, and the amplification and detection of the GOI and the reference gene can be analyzed as a singleplex or multiplex reaction. The multiplex RQ-PCR assay is a quick, sensitive, reproducible, and cost-effective method for an accurate assessment [23]. Multiplex RQ-PCR in a routine practice enables an increase in throughput and reliability, with a reduction in pipetting errors. The sensitivity of this method is about 0.01% of the minor genotype. Due to the influence of the so-called Monte Carlo effect (a greater degree of random effect on very low percentages), it is appropriate to define the lowest significant detection limit of the method. On the other hand, the RQ-PCR is suitable to use for the quantification of up to about 10% of the minor genotype, since it has a lower accuracy at higher percentages.

3. Standard procedures for the monitoring of cell chimerism at the Institute of Hematology and Blood Transfusion

3.1 Informativity determination

The informativity determination always precedes the monitoring of cell chimerism. Recipient and donor DNAs are tested by a panel of highly polymorphic STRs and InDels by multiplex kits. VNTRs and SNPs are not used in our laboratory. Currently, the PowerPlex 16HS System (Promega, Madison, WI, USA) kit is used routinely for STR analysis; the kit contains 13 basic Combined DNA Index System (CODIS) core STR loci [24], sex-specific locus amelogenin, and 2 other pentanucleotide repeat polymorphisms Penta D and Penta E. For the genotyping of deletion–insertion polymorphisms (DIP), a Mentype DIPscreen (Biotype, Dresden, DE) kit is used that amplifies 33 DIP loci and amelogenin. A comparison of the donor's and the recipient's DNA profiles allows to select the specific informative markers suitable for the monitoring of cell chimerism during the posttransplantation course. We choose at least two informative polymorphisms located at different chromosomes specific for the recipient. It is necessary to take into account the potential cytogenetic changes that are associated with different types of cancer (such as genome instability, loss of heterozygosity, and chromosomal changes) [25, 26]. Only informative recipient alleles by at least n ± 2 repeats outside stutter region (preferably by 2–4 longer) are used for calculation. An artifact of PCR, the so-called DNA stutter, is a result of strand slippage during DNA synthesis, showing up primarily one repeat before and, less frequently, one repeat after the true allele. The result of quantification is that such an allele would lead to an incorrect interpretation. It is always appropriate to quantify the minor genotype; therefore, in the

case of graft rejection or graft failure (the donor's cells are present in <50%), we choose two informative polymorphisms specific for donor in the same way as the recipient.

3.2 Interpretation of chimerism status

Chimerism is a dynamic process, so the proportion of autologous cells after allo-HSCT can change during monitoring. It is therefore necessary to approach each patient individually and to select appropriate methods for quantification.

An overview and definition of the chimerism status are given in **Table 2**. Under optimal conditions, we can detect only the donor's genotype after allo-HSCT; thus, the recipient's hematopoiesis is completely replaced with the donor's graft. We interpret this as a complete chimerism (CC). In our laboratory the CC is detected by RQ-PCR, and, based on clinical validation, the significant detection limit was defined as ≤0.035% of the recipient's genotype (due to Monte Carlo effect as mentioned in point 2.2.3). The detection of recipient's genotype of less than 1% is interpreted as a microchimerism (range from ≥0.036 to <1%), and the presence of more than or equal 1% is interpreted as mixed chimerism (MC). The percentages of the individual alleles are then quantified by STR analysis. If we detect only the recipient's genotype or the donor's genotype less than 0.035%, transplant rejection and a complete recovery of the original hematopoiesis have occurred. Split chimerism can be seen in the analysis of cell fractions. This means that MC is detected in a certain leukocyte line, but in another cell line, it is CC. The analysis of cell subpopulations makes it possible to distinguish between residual malignant cells and nonmalignant hematopoiesis. More often, the fraction of monocytes, granulocytes, natural killer (NK) cells, T lymphocytes, and B lymphocytes is examined. The analysis of chimerism of T lymphocytes and NK cells can help, especially, as a guide to additional therapy in order to avoid graft rejection. The analysis of chimerism in cell fractions is particularly important for patients with a reduced intensity regimen before allo-HSCT or patients with autoimmune disease.

The interpretation of bone marrow samples is more difficult. Microchimerism is often detected as a result of the contamination of the primary sample by bone marrow stromal cells of the recipient. Therefore, microchimerism below 0.5% of the recipient's genotype is considered to be insignificant. The proportion of autologous hematopoiesis in the bone marrow can fluctuate over time, especially in the early period after allo-HSCT. In making a clinical decision, it is more important to watch the dynamics of chimerism and take into account the patient's diagnosis rather than the individual values of microchimerism or MC.

Chimerism status	Definition
Complete chimerism (CC)	Detection of donor's genotype only or ≤0.035% recipient's genotype
Microchimerism	Detection of donor/recipient ratio ≥0.036 to <1%
Mixed chimerism (MC)	Detection of donor/recipient ratio ≥1%
Split chimerism	Mixed chimerism is detected in a certain leukocyte line, but in another cell line, it is complete chimerism
Autologous hematopoiesis	Detection of recipient's genotype only or ≤0.035% donor's genotype

Table 2.
Interpretation of chimerism status.

3.3 Posttransplant monitoring

The monitoring of cell chimerism consists of analyzing 2–3 selected informative polymorphisms. The frequency of monitoring after graft transfer is performed under our standard days (D) +14, +21, and +28 after allo-HSCT in both adult and pediatric patients. Subsequently, in pediatric patients, the intervals of examination are every 2–3 weeks up to D + 180, once a month to the first year after allo-HSCT, later every 2–4 months up to 3 years after allo-HSCT, and every 6–12 months up to 5 years after allo-HSCT according to the dates of outpatient's controls. In adult patients, examinations from the second month after allo-HSCT are carried out in monthly intervals up to 2 years and, throughout the next period, at least every 6 months. The frequency of examinations depends on the patient's medical condition, diagnosis, the dynamics of their chimerism status, and especially the physician's decision. In cases of increasing microchimerism or MC detection after the previous period of CC, an intensive investigation scheme is recommended due to the risk of graft rejection or relapse of the primary disease.

In the first samples after allo-HSCT, the detection of MC can be expected. If the recipient's genotype fraction falls below 50%, we can interpret this as the so-called engraftment of the donor's cells. The MC gradually decreases until the patient reaches CC. The median achievement of CC is most often D + 21 or D + 28 after allo-HSCT and depends on the patient's diagnosis and many other factors such as the regimen of allo-HSCT or the quality of the graft.

The choice of method used depends on the patient's actual chimerism status. Due to the high sensitivity of the RQ-PCR method, in combination with STR analysis, it is advisable to use RQ-PCR for the monitoring of patients in cases where CC, microchimerism, or MC up to 10% has been detected. On the other hand, with a rising trend of MC, it is better to use only STR analysis for quantification. For more accurate determination, it is always necessary to analyze the informative polymorphisms that detect a minority genotype. This means that in cases where the MC increases over 50% of the recipient's genotype, it is preferable to select donor-specific polymorphisms for quantification.

3.4 The importance of microchimerism

The introduction of the RQ-PCR method for the monitoring of cell chimerism as a part of routine examination has improved significantly the sensitivity of the assessment. Its high sensitivity of 0.035% allows for a much earlier detection of relapses than conventional methods (VNTR and STR). The importance of microchimerism detection was confirmed by us and many other studies [27–30], and we would like to present our retrospective data below.

3.4.1 Patients and methods

A group of 224 patients, from HLA-identical-related and HLA-identical-unrelated donors, who underwent allo-HSCT between 2011 and 2015 at the Institute of Hematology and Blood Transfusion, were enrolled in this study. Patients with early HSCT-associated mortality (less than 14 days), another allo-HSCT before the third year, with no RQ-PCR analyses or with a loss of follow-up were excluded. In total, 207 patients were eligible for analysis of cell chimerism dynamics. The test group was divided into 3 subgroups according to chimerism status 3 years after allo-HSCT: patients with CC (137), patients with microchimerism (38), and patients with MC (32). The patients' characteristics are listed in **Table 3**.

	Number	%
Patient characteristic	207	100
Age		
Median: 53 years (range 20–67 years)		
Sex		
Male	130	63
Female	77	57
Diagnostic group		
AML	91	44.0
CML	9	4.3
MDS	22	9.6
ALL and LBL	20	9.7
Myeloproliferative disease and MDS/MPS	18	8.7
B-cell non-Hodgkin's lymphoma	10	4..8
CLL, SLL, PLL	17	8.2
Mature T-cell and NK-cell lymphomas	11	5.3
Hodgkin's lymphoma	2	1.0
Other diseases	7	3.4
Transplant characteristic		
Donor		
HLA match	207	100
Match family donor	64	31
Match unrelated donor	143	69
Conditioning regimen		
Myeloablative	126	61
Nonmyeloablative	81	39
Stem cell source		
PBPC	180	87
BM	27	13

Abbreviations: AML, acute myeloid leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; ALL and LBL, acute lymphoblastic leukemia and lymphoblastic lymphoma; MDS/MPS, myelodysplastic/myeloproliferative neoplasms; CLL, SLL, PLL, chronic lymphoblastic leukemia, small lymphocytic lymphoma, prolymphocytic leukemia; PBPC, peripheral blood progenitor cells; BM, bone marrow.

Table 3.
Patient characteristics.

DNA from whole peripheral blood samples was isolated by means of a salting-out procedure [31] and diluted to a final concentration of 50 ng/μL. The combination of InDels by RQ-PCR, in conjunction with STR analysis by fragment analysis, was used to determine the chimerism status. Fragment analysis of the resulting PCR products was performed on an automated 3500 Series Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA), and data were processed by GeneMapper v5 software (Thermo Fisher Scientific). Quantification of MC was determined using the peak areas representing specific alleles. InDel analysis was performed by means of TaqMan technology. Rotor-Gene machine (Corbett Life Science, Sydney, New

South Wales) and Rotor-Gene 6 software were used for evaluation. The percentage of microchimerism was calculated by the ΔΔCT method. Data were normalized with the glyceraldehyde-3-phosphate dehydrogenase as a reference gene.

The impact of chimerism status on the 3-year overall survival of allo-HSCT patients and 3-year relapse rates was evaluated using GraphPad Prism 7 software (La Jolla, CA, USA). The logrank (Mantel-Cox) test was used for comparison of survival curves.

3.4.2 Results

The 3-year overall survival probability (**Figure 1**) in our cohort was 59%, and a significant difference was found in all three subgroups: CC vs. MC ($p < 0.0001$); CC vs. microchimerism ($p < 0.0001$); and MC vs. microchimerism ($p = 0.0485$). Overall 85 patients died: the main cause of death was a relapse of the primary disease (24%); in the second it was pneumonia (20%); and in the third it was multiple organ failure (15%). Infections, acute GvHD, cerebrovascular accident, other pulmonary complications, and heart attack occurred less frequently. However, if we take into account the distribution of patients in the three subgroups according to their chimerism status, the main cause of death in the CC subgroup was pneumonia (24%), other infections (16%), and multiple organ failure (16%); the relapse was only 5% (two patients). In these patients, the last available sample was taken 2–3 months prior to the relapse date. In the microchimerism subgroup, the main cause of death was relapse (36%), the second most often was multiple organ failure (18%), and in the third, it was pneumonia (14%). In the MC subgroup, most patients died of a relapse (38%), followed by pneumonia (19%), and multiple organ failure (12%).

Overall, the relapse mortality was 67%. In a correlation of 3-year overall relapse rates (**Figure 2**), there was a significant difference between all subgroups: CC vs. MC patients ($p < 0.0001$), CC vs. microchimerism patients ($p = 0.0073$), and MC vs. microchimerism patients ($p = 0.0007$). Patients with MC relapsed in 72% of cases, and the subsequent mortality was 87%. The detection of MC, especially in the early period after allo-HSCT, is thus an important high-risk factor for a relapse of the disease. In the microchimerism subgroup, patients relapsed in 34% of cases with a mortality rate of 69%. The patients with microchimerism are considered to be potentially at risk. In the CC subgroup, patients relapsed in 15% of cases with a mortality rate of 29%.

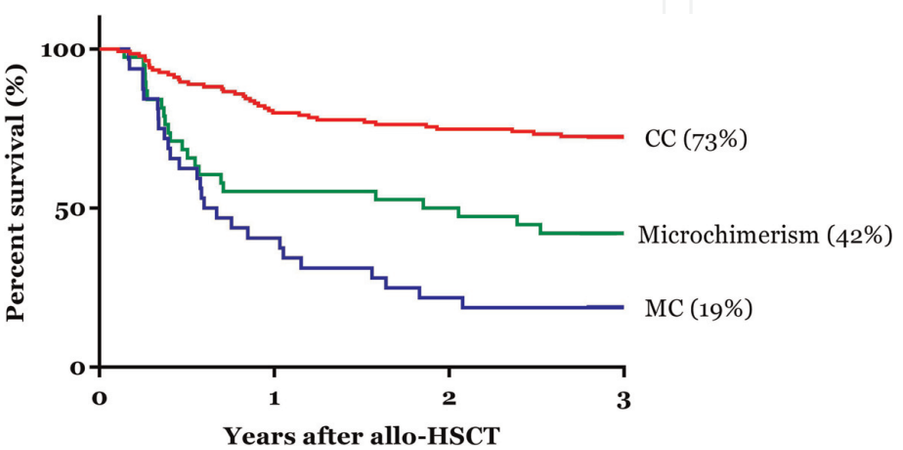


Figure 1.
Three-year overall survival probability according to chimerism status.

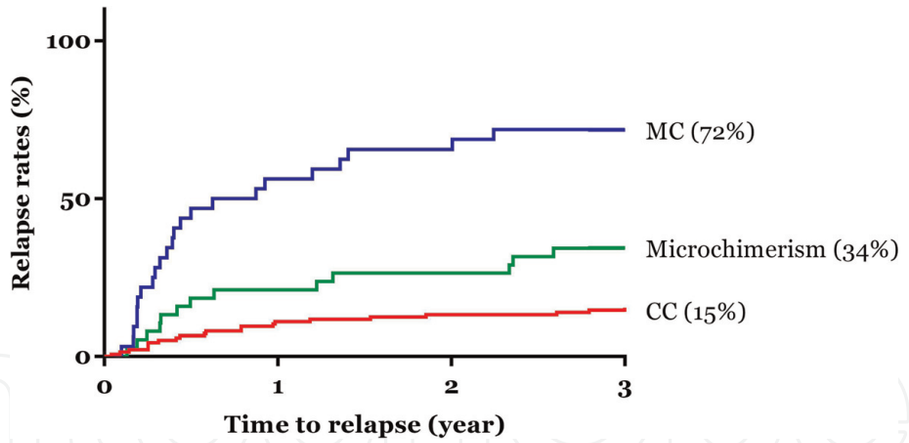


Figure 2.
Three-year overall relapse rates according to chimerism status.

4. Discussion

During the last decades, the effect of MC on the occurrence of imminent relapse has been investigated. At the beginning of the monitoring of cell chimerism by RFLP, red cell phenotyping, cytogenetic analysis, or VNTR analysis, there was no correlation found between the presence of MC and a relapse of the primary disease [32, 33]. Following the advances in methodology and the introduction of more sensitive methods for the monitoring of cell chimerism, scientists are increasingly convinced that there is a connection between the presence of MC and the incidence of relapse [34, 35]. These observations also support findings that studied the correlation of MC, minimal residual disease, and the presence of a relapse [36, 37]. Our results support this notion since we found a statistical significant difference between 3-year relapse rates and 3-year survival probability between all three subgroups based on their chimerism status. In addition, there were differences in the main causes of death: patients with MC and microchimerism died most often with a relapse, whereas CC patients died mostly of pneumonia.

In the group of patients with MC, it is also advisable to consider the issue of persisting MC (PMC). In the case of PMC, it depends on the diagnoses. In some diagnoses, PMC is typical and, usually, does not lead to a relapse (e.g., in nonmalignant diseases). In another diagnosis, PMC could be a problem; for instance, in MC patients who have a high percent of autologous cells immediately after the transplantation, it can often lead to a relapse, and the patients are often retransplanted. In our cohort of MC patients, there were eight patients without relapse. One of them relapsed shortly after 3 years, six patients died within first year after allo-HSCT from other causes, and one patient with primary myelofibrosis has never reached CC, and he is still around 1% of autologous hematopoiesis without recurrence of the disease 7 years after allo-HSCT.

The introduction of the RQ-PCR method for cell chimerism level monitoring as a part of routine examination has improved significantly the detection of microchimerism. The early identification of patients at risk is now possible, and, due to the early therapeutic intervention, we can avoid the progression to a high-risk category of MC. Thus, early detection of autologous hematopoiesis is essential for survival. In the case of MC or microchimerism detection, it is necessary to accurately quantify the proportion of recipient genotype and monitor its dynamics over time.

Recently, other studies have also begun to focus on the monitoring of cell chimerism using the latest technologies such as digital PCR [38] or next-generation

sequencing [39]. The incorporation of these techniques into routine investigations depends on subsequent validations that will reveal their advantages or disadvantages.

5. Conclusion

Molecular analysis of hematopoietic chimerism at a defined period after allo-HSCT represents a valuable non-specific marker of posttransplant course for all diagnoses. For some diagnoses this is the only available marker for monitoring. The aim of cell chimerism analysis is to provide a conclusive base for informed, clinical decision-making. The establishment of an adequate monitoring schedule, as well as the selection of appropriate markers and interpretation criteria, will improve the clinical value of this analysis. The use of sensitive methods, like RQ-PCR for the monitoring of cell chimerism, is important for the early detection of relapse and allows the early initiation of medical treatment.

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Conflict of interest

The authors declare no competing financial interests.

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References

[1] Anderson D, Billingham RE, Lampkin GH, Medawar PB. The use of skin grafting to distinguish between monozygotic and dizygotic twins in cattle. Heredity. 1951;**5**:379-397

[2] Bianchi DW, Zickwolf GK, Weil GJ, Sylvester S, DeMaria MA. Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. Proceedings of the National Academy of Sciences of the United States of America. 1996;**93**(2):705-708. DOI: 10.1073/pnas.93.2.705. PubMed PMID: 8570620; PubMed Central PMCID: PMC40117

[3] Yunis EJ, Zuniga J, Romero V, Yunis EJ. Chimerism and tetragametic chimerism in humans: Implications in autoimmunity, allorecognition and tolerance. Immunologic Research. 2007; **38**(1–3):213-236. Review. PubMed PMID: 17917028

[4] Chen CT, Gau JP, Liu JH, Chiou TJ, Hsiao LT, Liu YC. Early achievement of full donor chimerism after allogeneic hematopoietic stem cell transplantation predicts lower relapse risk in patients with acute lymphoblastic leukemia. Journal of the Chinese Medical Association. 2018;**81**(12):1038-1043. DOI: 10.1016/j.jcma.2018.06.005. Epub 2018 Aug 9. PubMed PMID: 30100353

[5] Shastry BS. SNP alleles in human disease and evolution. Journal of Human Genetics. 2002;**47**(11):561-566. Review. PubMed PMID: 12436191

[6] Harris H. Polymorphism and protein evolution. The neutral mutation-random drift hypothesis. Journal of Medical Genetics. 1971;**8**(4):444-452. Review. PubMed PMID: 4948373; PubMed Central PMCID: PMC1469095

[7] Bader P, Niethammer D, Willasch A, Kreyenberg H, Klingebiel T. How and when should we monitor chimerism after allogeneic stem cell transplantation? Bone Marrow Transplantation. 2005;**35**(2):107-119. Review. PubMed PMID: 15502849

[8] Khan F, Agarwal A, Agrawal S. Significance of chimerism in hematopoietic stem cell transplantation: New variations on an old theme. Bone Marrow Transplantation. 2004; **34**(1):1-12. Review. PubMed PMID: 15156163

[9] Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics. 1980;**32**(3):314-331. Review. PubMed PMID: 6247908; PubMed Central PMCID: PMC1686077

[10] Lawler M, McCann SR, Conneally E, Humphries P. Chimaerism following allogeneic bone marrow transplantation: Detection of residual host cells using the polymerase chain reaction. British Journal of Haematology. 1989;**73**(2): 205-210. PubMed PMID: 2818939

[11] Schreiner T, Prochnow-Calzia H, Maccari B, Erne E, Kinzler I, Wölpl A, et al. Chimerism analysis after allogeneic bone marrow transplantation with nonradioactive RFLP and PCR-AFLP using the same DNA. Journal of Immunological Methods. 1996;**196**(1): 93-96. PubMed PMID: 8841447

[12] Tautz D. Notes on the definition and nomenclature of tandemly repetitive DNA sequences. EXS. 1993;**67**:21-28. Review. PubMed PMID: 8400689

[13] Edwards A, Civitello A, Hammond HA, Caskey CT. DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. American Journal of Human Genetics. 1991;**49**(4): 746-756. PubMed PMID: 1897522; PubMed Central PMCID: PMC1683171

[14] The Utah Marker Development Group. A collection of ordered tetranucleotide-repeat markers from the human genome. American Journal of Human Genetics. 1995;**57**:619-628

[15] STRBase, National Institute of Standards and Technology. 2019. Available from: https://strbase.nist.gov/index.htm

[16] Ruitberg CM, Reeder DJ, Butler JM. STRBase: A short tandem repeat DNA database for the human identity testing community. Nucleic Acids Research. 2001;**29**(1):320-322. PubMed PMID: 11125125; PubMed Central PMCID: PMC29767

[17] Kayser M, de Knijff P, Dieltjes P, Krawczak M, Nagy M, Zerjal T, et al. Applications of microsatellite-based Y chromosome haplotyping. Electrophoresis. 1997;**18**(9):1602-1607. PubMed PMID: 9378129

[18] Weber JL, David D, Heil J, Fan Y, Zhao C, Marth G. Human diallelic insertion/deletion polymorphisms. American Journal of Human Genetics. 2002;**71**:854-862

[19] Mills RE. An initial map of insertion and deletion (INDEL) variation in the human genome. Genome Research. 2006;**16**(9):1182-1190. DOI: 10.1101/gr.4565806. PMC 1557762

[20] van der Velden VH, Hochhaus A, Cazzaniga G, Szczepanski T, Gabert J, van Dongen JJ. Detection of minimal residual disease in hematologic malignancies by real-time quantitative PCR: Principles, approaches, and laboratory aspects. Leukemia. 2003; **17**(6):1013-1034. DOI: 10.1038/sj.leu.2402922. Review. PubMed PMID: 12764363

[21] Alizadeh M, Bernard M, Danic B, Dauriac C, Birebent B, Lapart C, et al. Quantitative assessment of hematopoietic chimerism after bone marrow transplantation by real-time quantitative polymerase chain reaction. Blood. 2002;**99**(12):4618-4625. PubMed PMID: 12036896

[22] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 2001;**25**(4):402-408. DOI: 10.1006/meth.2001.1262. PubMed PMID: 11846609

[23] Nadvornikova S, Leontovycova M, Pegova K, Hrabakova P, Prerovska R, Cechova H. Multiplex real-time quantitative polymerase chain reaction assay for rapid and sensitive detection of hematopoietic chimerism. HLA. 2018;**92**(4):215-223. DOI: 10.1111/tan.13383. PubMed PMID: 30129247

[24] Butler JM. Genetics and genomics of core STR loci used in human identity testing. Journal of Forensic Sciences. 2006;**51**(2):253-265

[25] Fröhling S, Döhner H. Chromosomal abnormalities in cancer. The New England Journal of Medicine. 2008;**359**:722-734

[26] Bernheim A. Cytogenomics of cancers: From chromosome to sequence. Molecular Oncology. 2010;**4**(4): 309-322. DOI: 10.1016/j.molonc.2010.06.003. Epub 2010 Jun 11. Review. PubMed PMID: 20599448; PubMed Central PMCID: PMC5527907

[27] Koldehoff M, Steckel NK, Hlinka M, Beelen DW, Elmaagacli AH. Quantitative analysis of chimerism after allogeneic stem cell transplantation by real-time polymerase chain reaction with single nucleotide polymorphisms, standard tandem repeats, and Y-chromosome-specific sequences. American Journal of Hematology. 2006; **81**(10):735-746. DOI: 10.1002/ajh.20693. PubMed PMID: 16838323

[28] Horky O, Mayer J, Kablaskova L, Razga F, Krejci M, Kissova J, et al. Increasing hematopoietic microchimerism is a reliable indicator of incipient AML relapse. International Journal of Laboratory Hematology. 2011;**33**(1):57-66. DOI: 10.1111/j.1751-553X.2010.01249.x. PubMed PMID: 20681999

[29] Jacque N, Nguyen S, Golmard JL, Uzunov M, Garnier A, Leblond V, et al. Chimerism analysis in peripheral blood using indel quantitative real-time PCR is a useful tool to predict post-transplant relapse in acute leukemia. Bone Marrow Transplantation. 2015;**50**(2):259-265. DOI: 10.1038/bmt.2014.254. Epub 2014 Nov 10. PubMed PMID: 25387089

[30] Ahci M, Stempelmann K, Buttkereit U, Crivello P, Trilling M, Heinold A, et al. Clinical utility of quantitative PCR for chimerism and engraftment monitoring after allogeneic stem cell transplantation for hematologic malignancies. Biology of Blood and Marrow Transplantation. 2017;**23**(10):1658-1668. DOI: 10.1016/j.bbmt.2017.05.031. Epub 2017 Jun 8. PubMed PMID: 28603070

[31] Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research. 1988; **16**(3):1215

[32] Schattenberg A, De Witte T, Salden M, Vet J, Van Dijk B, Smeets D, et al. Mixed hematopoietic chimerism after allogeneic transplantation with lymphocyte-depleted bone marrow is not associated with a higher incidence of relapse. Blood. 1989;**73**(5):1367-1372. PubMed PMID: 2564791

[33] van Leeuwen JE, van Tol MJ, Joosten AM, Wijnen JT, Khan PM, Vossen JM. Mixed T-lymphoid chimerism after allogeneic bone marrow transplantation for hematologic malignancies of children is not correlated with relapse. Blood 1993; **82**(6):1921–1928. PubMed PMID: 8400242

[34] Lamba R, Abella E, Kukuruga D, Klein J, Savasan S, Abidi MH, et al. Mixed hematopoietic chimerism at day 90 following allogenic myeloablative stem cell transplantation is a predictor of relapse and survival. Leukemia. 2004; **18**(10):1681-1686. DOI: 10.1038/sj.leu.2403468. PubMed PMID: 15318247

[35] Cechova H, Leontovycova M, Pavlatova L. Chimerism as an important marker in post-transplant monitoring chimerism monitoring. HLA. 2018;**92** (Suppl 2):60-63. DOI: 10.1111/tan.13407. PubMed PMID: 30362266

[36] Formánková R, Honzátková L, Moravcová J, Sieglová Z, Dvořáková R, Nádvorníková S, et al. Prediction and reversion of post-transplant relapse in patients with chronic myeloid leukemia using mixed chimerism and residual disease detection and adoptive immunotherapy. Leukemia Research. 2000;**24**(4):339-347. PubMed PMID: 10713331

[37] Terwey TH, Hemmati PG, Nagy M, Pfeifer H, Gökbuget N, Brüggemann M, et al. Comparison of chimerism and minimal residual disease monitoring for relapse prediction after allogeneic stem cell transplantation for adult acute lymphoblastic leukemia. Biology of Blood and Marrow Transplantation. 2014;**20**(10):1522-1529. DOI: 10.1016/j.bbmt.2014.05.026. Epub 2014 Jun 4

[38] Valero-Garcia J, González-Espinosa MC, Barrios M, Carmona-Antoñanzas G, García-Planells J, Ruiz-Lafora C, et al. Earlier relapse detection after allogeneic hematopoietic stem cell transplantation by chimerism assays: Digital PCR versus quantitative real-time PCR of insertion/deletion polymorphisms. PLoS One. 2019;**14**(3): e0213966. DOI: 10.1371/journal.pone.0212708

[39] Kim J, Hwang IS, Kim HS, Joo DJ, Hong KR, Choi JR. Bone marrow chimerism detection using next generation sequencing based on single nucleotide polymorphisms following liver transplantation: Comparison with short tandem repeat-PCR. Annals of Laboratory Medicine. 2016;**36**(1):82-84. DOI: 10.3343/alm.2016.36.1.82. PubMed PMID: 26522768; PubMed Central PMCID: PMC4697352

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