https://doi.org/10.23873/2074-0506-2022-14-4-488-499

Monitoring of chimerism after allogeneic hematopoietic stem cell transplantation

D.S. Dubnyak✉, N.V. Risinskaya, M.Yu. Drokov, A.B. Sudarikov

National Medical Research Center for Hematology,

4 Noviy Zykovskiy Dr., Moscow 125167 Russia

✉Corresponding author: Darya S. Dubnyak, Hematologist, National Medical Research Center for Hematology, darya-dubnyak@yandex.ru

Abstract

Allogeneic hematopoietic stem cells transplantation is one of the effective methods of treating patients with diseases of the blood system.

Establishment of complete (100%) donor chimerism is among of the main indicators of successful transplantation in such cases. Monitoring chimerism makes it possible both to assess the graft acceptance, and also potentially predict the risk of developing primary/secondary graft failure, relapse, and graft-versus-host disease.

The purpose of this review is to summarize the main concepts associated with chimerism after allogeneic hematopoietic stem cell transplantation; consideration of the need to study chimerism in various cell populations, as well as the relationship between chimerism and the development of various immunological complications.

Keywords: allogeneic hematopoietic stem cell, transplantation, chimerism after transplantation

Conflict of interests. Authors declare no conflict of interest.

Financing. The study was performed without external funding.

For citation: Dubnyak DS, Risinskaya NV, Drokov MYu, Sudarikov AB. Monitoring of chimerism after allogeneic hematopoietic stem cell transplantation.

Transplantologiya. The Russian Journal of Transplantation. 2022;14(4):488–499. (In Russ.). https://doi. org/10.23873/2074-0506-2022-14-4-488-499

aGVHD, acute graft-versus-host disease
allo-HSCT, allogeneic hematopoietic stem cell transplantation
DNA, deoxyribonucleic acid
dPCR, digital droplet polymerase chain reaction
FISH, fluorescence in situ hybridization
In/del, insertion/deletion (polymorphism)
MRD, minimal residual disease
PCR, polymerase chain reaction
qPCR, quantitative real-time PCR assay
SNP, single nucleotide polymorphism
STR, short tandem repeat
VNTR, variable number tandem repeat

History of chimerism research

The coexistence of cells of more than one genetic origin in one individual is called a biological chimera. Chimerism is assessed by the proportion of deoxyribonucleic acid (DNA) isolated from blood and/or bone marrow cells belonging to the donor and recipient after transplantation of allogeneic hematopoietic stem cells (alloHSCT). The first researcher to describe the phenomenon of chimerism was Ray Owen [1]. In 1945, he first showed mixed chimerism in dizygotic twin calves. As it turned out, in the bloodstream of calves there were both the autologous erythrocytes, and also the erythrocytes of the twin, which entered the body during intrauterine life.

Subsequently, Peter Medawar and colleagues demonstrated immunological tolerance in dizygotic twin calves [2]. In their study, they performed skin grafting on cattle and recorded the acceptance of the grafts without immunosuppressive therapy. For the first time in transplantology, the term "chimera" was used by S. Ford and colleagues.

In 1956, the researchers published a paper describing the administration of allogeneic hematopoietic cells to mice after irradiation [3].

In 1959, Thomas and colleagues performed the first successful transplantation in two patients with acute lymphoblastic leukemia. Patients underwent total body irradiation followed by bone marrow infusion. Parameters were restored after 2 weeks. However, after a few months, patients died from a relapse of the disease [4]. Currently, monitoring of chimerism is a mandatory study in patients after alloHSCT. The basis for determining chimerism is the analysis of genetic differences between donor and recipient cells using various investigation techniques [5].

Overview of investigation techniques for chimerism

In the molecular genetic analysis of chimerism, the obligatory first step before performing alloHSCT is the isolation of DNA from peripheral blood cells or bone marrow of the recipient and donor to determine genetic profiles [6].

One of the first methods of routine monitoring of chimerism was the study of erythrocyte antigens using the hemagglutination test. The method is considered simple and reproducible, however, it should be noted that in the early stages after alloHSCT, this diagnostic method should not be relied upon, given the long-term persistence of erythrocytes belonging to the recipient, as well as the frequent coincidence of the donor-recipient pair by blood type. Currently, the method is not used in routine clinical practice to monitor chimerism, since it does not allow the study of chimerism in "clinically significant" cell populations [7].

Another method that has been used to monitor chimerism is flow cytometry analysis. The chimerism was studied using monoclonal antibodies directed against AB0 and C, c, D, E, e erythrocyte antigens.

The disadvantage of the method is that the study can be carried out only in situations where the recipient did not receive blood transfusion therapy, this method is not applicable in assessing chimerism in other cell populations [8, 9].

Currently, in clinical practice, fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR) are most in demand as methods for monitoring chimerism.

Chimerism is monitored by FISH using specific probes for sex chromosomes followed by fluorescence microscopy. The sensitivity of the detection of the minor genotype makes 0.4–5% [10, 11]. The main disadvantage of the method is that it is impossible to perform the test if the donor-recipient pair is of the same sex [12, 13], therefore, in 50% of cases, this method for diagnosing chimerism is not applicable [14].

When monitoring chimerism using PCR, the polymorphic regions of the genome are studied, which makes it possible to distinguish between the donor and recipient alleles. This assay is based on the amplification variable number tandem repeat (VNTRs), short tandem repeats (STRs), single nucleotide polymorphisms (SNPs), insertion/deletion polymorphisms (In/del). Its important advantage is the possibility of using it in all donor-recipient pairs, with the exception of identical twins. [15].

Subsequently, the STR analysis due to its high informative value became the gold standard for determining chimerism. STRs are repeatedly and sequentially repeated DNA fragments from 1 to 6 bases in length. STR loci occupy up to 3% of human genomic DNA and are mainly located in its non-coding regions. Owing of their high polymorphism due to evolutionarily neutral errors in replication folds resulting in multiple allelic variants, STRs are widely used in biological and medical research. Currently, multiallelic spectra are well studied -

allele variants (approximately from 3 to 50 units of one repeat) of more than 5 thousand STR loci, and the most versatile informative STR panels suitable for both forensic medicine and chimerism monitoring have been selected. [16–18]. The principle of multiplex STR-PCR method implies the selection of loci with the same amplification conditions and different DNA-target lengths. Primers for these loci are tagged with fluorescent dyes, and the amplified product is fractionated by capillary electrophoresis. The fluorescence detector registers the intensity of the signal from each amplicon, and the addition of a dimension standard to the samples then allows, by using software, to obtain an STR profile consisting of a number of amplicons of the calculated length. A relative quantitative analysis of the proportion of "recipient/donor" markers in the sample reflects the proportion of "recipient/donor" nuclear cells in the studied material of the patient after allo-HSCT. Unique markers for determining chimerism are identified by comparing the STR profiles of the recipient and the donor. If the STR profiles of the twins match completely, a conclusion is made about the syngeneity of the donor and the recipient, and the impossibility of monitoring the post-transplantation chimerism by STR profiling. In all other cases, post-transplant chimerism is calculated as the mean of the fluorescent signal of the recipient unique STR markers from the total fluorescent signal for each informative locus [19]. A small amount of DNA ~1–5 ng is required for the primary study, however, for the detection of minor DNA in samples analyzed for chimerism, the amount of DNA must be increased [20]. The sensitivity of this method is 1–5%, which is rather low, since it is based on a competitive reaction with the same primers to the DNA targets that differ in length. The method is informative, reproducible in cross-laboratory studies, does not require the construction of calibration curves or setting

controls for each subsequent monitoring; that is why it is widely spread in clinical practice [5].

There are other methods for monitoring chimerism using the quantitative PCR method. M. Alizadeh et al. proposed a method based on quantitative real-time PCR (qPCR) for the detection of single nucleotide polymorphisms (SNPs) or short deletions/insertions (In/del) [11]. The panel proposed by Alizadeh includes from 20 DNA targets, however, in a case of related transplantation, this panel may not be enough to identify unique markers of the recipient and donor chimerism. In this case, the panel can be expanded with all the test systems available in the arsenal of the laboratory for the detection of allelic polymorphisms by the qPCR method. This method of monitoring chimerism is highly sensitive due to the use of allele-specific primers. The sensitivity of qPCR is 0.1% [21, 22].

With the development of new technologies, an SNP analysis method with even higher sensitivity from 0.01% for minor DNA has been proposed. This is a digital droplet PCR (dPCR), a method for the absolute quantification of DNA targets. In dPCR, the reaction mixture, after adding DNA, is broken into many microdroplets that fall into the cells of a special chip, each of which is PCR with specific primers. Wells containing amplified target sequences are detected by fluorescence. The proportion of PCR-positive cells is sufficient to determine the concentration of the target sequence without the need for calibration [23]. It is also possible to conduct dPCR directly in the droplet emulsion, which greatly simplifies the analysis [24]. It should be noted that this method has limitations in clinical practice, since the study requires a minimum amount of DNA ~ 50-75 ng, which is often impossible, given that most patients after alloHSCT have cytopenia [25]. The method is suitable for monitoring the minimal residual disease, but the question

remains whether such a high sensitivity is needed in the study of chimerism. When taking blood or bone marrow, the sample will necessarily contain a small admixture (up to 0.1%) of patient's own cells, and everything that is higher can be determined by routine qPCR and STR-PCR methods. In addition, the sensitivity of routine methods for monitoring chimerism can be greatly increased by examining the chimerism in those selected cell populations, which include the patient's tumor cells. Table 1 shows the advantages and disadvantages of the main current molecular methods for studying chimerism.

Table 1. Main methods for studying chimerism in patients after allogeneic hematopoietic stem cell transplantation [6–14, 22–25]

Method name	Advantages	Flaws
Study of erythrocyte antigens (hemagglutination test)	Reproducibility	Study of only one cell population
Cytoflowmetric analysis	Reproducibility	The method is not applicable if blood transfusion therapy was performed. Study of chimerism in a single cell population
Fluorescence in situ hybridization (FISH)	Informative	Impossible to assess chimerism in patients and donors of the same gender
STR-PCR	Informative, reproducible	Low sensitivity of 1–5%
qPCR	High sensitivity of the method 0.1%	Limitations in transplantation from a related donor
dPCR	Very high sensitivity of the method 0.01%	The need for high cellularity of the sample to perform the study

Key concepts assessing chimerism after allogeneic hematopoietic stem cell transplantation

After successful alloHSCT, complete donor chimerism is established in the bone marrow/peripheral blood.

Complete donor chimerism implies the detection of more than 95−99.9% of cells in the bone marrow/peripheral blood that have the

donor genotype. Mixed chimerism implies the detection of 5–95% cells of host origin in bone marrow/peripheral blood [26]. There is the concept of "split chimerism", when among the total population of bone marrow cells, peripheral blood, complete donor chimerism is observed; and mixed chimerism is seen in various individual cell populations [27, 28]. There are also some studies, in which the mixed chimerism is classified separately into transient, progressive and stable mixed chimerism. In case of transient mixed chimerism, a mixed population of donor and recipient cells is identified within a year after alloHSCT, with further confirmation of complete chimerism. If the population of cells belonging to the recipient increased by more than 15% within 3 months, then such mixed chimerism was called progressive. In stable mixed chimerism, less than 5% of cells with the host genotype are identified over a long period of time [29, 30]. Table 2 shows the possible statuses of the patient based on studying the chimerism.

Table 2. Evaluation of chimerism in patients after allogeneic hematopoietic stem cell transplantation [26, 29, 30]

State	Number of cells with donor DNA	Number of cells with host DNA	Evaluation
Complete donor chimerism	> 95–99.9%	< 5–0.1%	At the time of study
Mixed donor chimerism	5–95%	95–5%	At the time of study
Stable mixed chimerism	5–95%	95–5%	Within 3 months
Transient mixed chimerism	5–95%	95–5%	Increase in host DNA-containing cells by 15% within 3 months

Temporal protocol for monitoring chimerism

With a stable clinical pattern, most transplant centers monitor chimerism in combination with other diagnostic methods, starting from day 28 after allo-HSCT. Both the bone marrow aspirate and whole blood,

as well as isolated individual populations of blood cells and/or bone marrow, can serve as the material for the study. The duration of monitoring for chimerism after alloHSCT varies greatly at each transplant center. Follow-up for 5 years is recommended after alloHSCT, however, each transplant center independently approves the recommended follow-up time [31]. Table 3 shows options for monitoring chimerism in patients after alloHSCT with regard to the clinical situation and the possibilities of selecting individual populations [32].

Table 3. Algorithm for monitoring chimerism after allogeneic hematopoietic stem cell transplantation

Target	Patients	Cell populations	Method	Time periods
Assessment of engraftment	All	T cells, NK cells of peripheral blood	STR-PCR	From the 15th day of alloHSCT: once in 14 days until stating complete chimerism
		Bone marrow aspirates	STR-PCR	Day 30 of alloHSCT
GVHD	All	T cells	STR-PCR	Once in 14 days until stating complete chimerism
Monitoring after ascertaining complete chimerism	Patients with non-malignant diseases of the blood system	Peripheral blood or bone marrow	STR-PCR	90, 180, 365 days of alloHSCT
Monitoring after ascertaining complete chimerism	Patients with malignant diseases of the blood system with the MRD marker	Peripheral blood	STR-PCR	Monthly for 1 year after alloHSCT; once every 3 months for the second year
		Bone marrow aspirates	STR-PCR	Once in 3 months within a year after alloHSCT
	Patients with malignant diseases of the blood system without the MRD marker*	Peripheral blood	qPCR	Monthly for 1 year after alloHSCT; once every 3 months for the second year
		Bone marrow aspirates	qPCR	Once in 3 months within a year after alloHSCT

Notes: *high sensitivity of the method for assessing chimerism allows MRD monitoring using this method; however, any blood or bone marrow sample will always contain some cells from the patient (e.g., epithelial cells), making it difficult to interpret the results of the study in patients without other markers of MRD. MRD, minimal residual disease

The choice of material for studying chimerism in patients after allogeneic hematopoietic stem cell transplantation

It should be noted that most transplant centers prefer to use peripheral blood as a material for the test as it implies a less invasive sampling technique compared to the bone marrow puncture [20]. However, in rare cases, blood and bone marrow chimerism may differ in some patients. For example, in their study J. Stump et al. compared chimerism in the bone marrow and peripheral blood in patients diagnosed with acute leukemia and other blood system diseases after alloHSCT [33]. Interestingly, 8 of 49 patients had differences in chimerism between bone marrow and peripheral blood. Thus, no cells belonging to the recipient were found in the peripheral blood; and 1–18% of the cells in the bone marrow, had the host genotype. In 2012, C.A. Rauwerdinck et al. published a retrospective study comparing chimerism in bone marrow and peripheral blood, including 42 patients. The investigators found no differences in chimerism rates between the two samples at 30, 60, and 90 days after alloHSCT [34]. Bach et al. in their study investigated chimerism using the real-time PCR assay. The difference in bone marrow and peripheral blood chimerism averaged 1.9% [35].

Relationship between chimerism and major events after allogeneic hematopoietic stem cell transplantation

Detecting mixed chimerism in malignant diseases of the blood system is associated with lower disease-free and overall survival of patients [36−38]. There are studies demonstrating that mixed chimerism at early stages after alloHSCT, namely in the first 3 months after transplantation, is not associated with the disease relapse [39]. A large retrospective study that included 688 patients was published in 2014. The work showed that the donor chimerism, comprising less than 90% of

cells, was associated with an increased risk of disease relapse [40]. A study by Bacher et al. demonstrated that in patients who achieve complete donor chimerism, a 3-year chance of relapse-free survival made 60%, in contrast to the group of patients with mixed chimerism, in whom a 3-year relapse-free survival was about 30% [41].

In recent years, more and more transplant centers have been studying both in bone marrow/peripheral blood aspirates, and also in individual cell populations (the so-called linear chimerism), which makes it possible to better assess the clinical pattern and predict possible complications at an early stage. To monitor chimerism in myeloid cells, CD15+, CD33+ cells are studied; CD3+ cells are studied to monitor chimerism in T lymphocytes; and CD19+ cells are studied for chimerism in B lymphocytes, followed by PCR. Less commonly, chimerism is assessed in NK (CD16/CD56) or CD71+ erythroid cells [31].

In their study L. Mountjoy et al. have shown that on the 30th and 60th days of alloHSCT, mixed chimerism in both lymphoid (CD3+) and myeloid (CD33+) cells is not associated with a low relapse-free or overall survival [42].

In a study by J. Deeg et al. an analysis of chimerism was made in patients with myelofibrosis after alloHSCT. The study included 131 patients. The results of the study demonstrated that the mixed CD33+ chimerism was associated with disease relapse [43].

So, according to S. Breuer et al., an early assessment of NK-cell chimerism may be a predictor of the graft failure development [44]. The studies by F. Baron, M. Bornh et al. have also demonstrated that the decreased donor genotype in NK cells below 50–75% was associated with a higher risk of graft failure [45–46].

F. Rosenow et al. investigated chimerism in patients with acute leukemia and myelodysplastic syndrome. A 3-year relapse-free survival

rate in patients with complete donor CD34+ cell chimerism was 74% versus 40% in patients with mixed chimerism [47].

According to Y. Jiang, Y.-N. Yang et al., in patients with B-acute lymphoblastic leukemia, a decrease in donor chimerism in B-lymphocytes is a predictor of disease relapse [48, 49]. In turn, the detection of mixed chimerism in a population of cells belonging to the myeloid lineage is usually a predictor of the graft rejection and/or the development of the disease relapse. [50, 51].

The detection of mixed T-cell chimerism may also be a predictor of disease relapse. According to the literature, a decreased proportion of the donor genotype T cells to 88.5% is associated with the development of disease relapse ($p=0.04$) [52]. Another study showed that donor T-cell chimerism at a level of 85% or less was associated with the development of relapse in patients after alloHSCT ($p=0.02$) [53].

If disease recurred against the mixed chimerism in the bone marrow and among different cell populations, then acute graft-versus-host disease (aGVHD) occurs, as a rule, amid the complete donor chimerism [54, 55].

According to the literature, it is the complete donor chimerism among T lymphocytes after alloHSCT that is a prognostic factor for the development of aGVHD. In the study by Baron, it was shown that an increase in the proportion of donor hematopoiesis of more than 90% T cells on the 28th day after alloHSCT is associated with the aGVHD development ($p=0.02$) [56]. Similar results were obtained by Antin; if by the 30th day after alloHSCT more than 90% among T cells had belonged to the donor genotype, aGVHD developed in 68% of cases compared to the group of patients who had had less than 90% of T cells of donor origin ($p=0.007$) [57].

In a retrospective study including 150 patients after allo-HSCT, it was noted that 25% of patients developed aGVHD. A detailed analysis of all patients with aGVHD of grade 2 and higher revealed the complete donor chimerism among T-cell populations on the 120th day after alloHSCT [54].

Table 4 reflects the relationship of mixed chimerism in selected populations with the development of complications after alloHSCT.

Table 4. Mixed chimerism in selected populations and association with an adverse event after allogeneic hematopoietic stem cell transplantation [43-49, 52, 53]

Population	Relation to an event
NK cells	Graft failure
CD34+ cells, CD33+ cells, B lymphocytes, T lymphocytes	Disease relapse

By monitoring chimerism in a particular population, it is also possible to trace the recovery of certain cell populations. So, in their study, I.V. Zvyagina et al. demonstrated a recovery of T-cell immunity in children after transplantation with the depletion of TCRαβ/CD19. The investigators showed that on the 60th day of alloHSCT, αβT cells have the genotype belonging to the recipient [58].

It should be taken into account that the isolation of individual cell populations is a laborious process that requires additional time for the assay, and the results obtained should be interpreted taking into account the dynamics of reconstitution among various cell subpopulations [59, 60].

Conclusion

Currently, the predictive potential of studying chimerism in various subpopulations has not been understood to the full, but it seems that chimerism monitoring in individual cell populations is more informative than chimerism in the total population of cells in patients with malignant blood diseases. The study of chimerism in individual cell populations can complement the study of chimerism in general, as well as help to prevent/predict various immunological complications after allogeneic hematopoietic stem cell transplantation, so to earlier use such options as the withdrawal of immunosuppressive therapy, transfusion of donor lymphocytes and allogeneic hematopoietic stem cell retransplantation.

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Information about the authors

Darya S. Dubnyak, Hematologist, National Medical Research Center for Hematology, https://orcid.org/0000-0002-2253-9870

30%, writing the text of the manuscript, analysis of publications on the topic of the article

Natalia V. Risinskaya, Cand. Sci. (Biol.), Senior Researcher, Laboratory of Molecular Hematology, National Medical Research Center for Hematology, https://orcid.org/0000-0003-2957-1619

25%, development of the concept and design of the review, editing the manuscript

Mikhail Yu. Drokov, Cand. Sci. (Med.), Hematologist, Head of the Sector for Scientific Research in Chemoblastosis Chemotherapy, Hematopoietic Depressions, and Bone Marrow Transplantation, National Medical Research Center for Hematology, https://orcid.org/0000-0001-9431-8316

25%, development of the concept and design of the review, editing the manuscript

Andrey B. Sudarikov, Dr. Sci. (Biol.), Head of the Laboratory of Molecular Hematology, National Medical Research Center for Hematology, https://orcid.org/0000-0001-9463-9187

20%, editing the manuscript

The article was received on September 8, 2022;
approved after reviewing September 27, 2022;
accepted for publication September 28, 2022