mebm
MOLECULAR AND EXPERIMENTAL BIOLOGY IN MEDICINE

METHODOLOGICAL ARTICLE

QUANTITATIVE PCR TECHNOLOGY IN CHIMERISM STATUS EVALUATION AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

Katarina Stingl Jankovic

Abstract: Chimerism status evaluation is one of the most useful methods for the assessment of the hematopoietic stem cell transplantation outcome, since it gives information about engraftment and disease relapse, as well as providing indication for timely interventions such as donor lymphocyte infusions. The aim of this article is to give an overview of the different methods used for chimerism detection, with a especial emphasis on the quantitative PCR (qPCR) based techniques as the newest in line of techniques used for chimerism monitoring. This overview covers a description of the qPCR method as well as a discussion about the advantages this technique offers in comparison to other methods.

[1]*Tissue Typing Centre, Clinical Department of Transfusion Medicine and Transplantation Biology, University Hospital Centre Zagreb, Zagreb, Croatia*

Corresponding author:
Katarina Stingl Jankovic
Tissue Typing Centre, Clinical Department of Transfusion Medicine and Transplantation Biology, University Hospital Centre Zagreb, Kispaticeva 12, 10000 Zagreb, Croatia
Tel: +385 1 23 67 287; Fax: + 385 1 23 67 337
e-mail: kstingl@kbc-zagreb.hr

Submitted: November, 2018
Accepted: January, 2019

Key words: chimerism, hematopoietic stem cell transplantation, quantitative PCR

INTRODUCTION

The assessment of the success of a medical treatment is always of great importance, and hematopoietic stem cell transplantation (HSCT), as a method of choice for treatment of numerous hematologic diseases and disorders, is no exception in this regard. The engraftment process and the detection of disease relapse are two key events which need to be monitored in post-HSCT patient follow-up procedures. Minimal residual disease (MRD) detection is an ideal procedure for the purpose of relapse detection because it reveals the presence of, for example, remaining leukemia cells.[1] Unfortunately, specific markers have not been discovered for all diseases and alternative methods have been developed in order to bypass the time period until such markers become available for diagnostic purposes. One such method is the evaluation of chimerism status after HSCT, which can also give valuable information about the state of donor cells engraftment.[2]

Chimerism, by medical definition, is a state in which two genetically different types of cells co-exist in one individual. It can happen naturally, for example after pregnancy, when maternal cells can be discovered in the child's circulation after birth and vice versa. Conversely, chimerism occurs as a consequence of certain medical procedures such as transfusion and transplantation.[3, 4]

The aim of HSCT is to replace the patient's hematopoietic system with donor's cells, either because the patient's cells do not function properly or have become malignant. If the main goal of the treatment is to restore the function of a cell line, such as for instance the T and B cell function in severe combined immunodeficiency (SCID) patients, then a complete replacement is not necessary, and a mixed chimerism, with both patient's and donor's cells detectable after transplantation, is sufficient for achieving adequate immune function. However, in cases when HSCT is performed in order to treat a malignant disease such as

acute myelogenous leukemia (AML), a complete conversion to a donor's hematopoietic system is essential because the malignant potential of the remaining patient' cells can be a cause for a disease relapse in the post-HSCT period. The accurate assessment of the chimeric status or, more precisely, determination whether the cells in the peripheral blood or bone marrow of the patient after transplantation are of patient or donor origin, is therefore of extreme importance.[5]

Initial protocols for HSCT almost always included myeloablative conditioning regimen in order to eradicate a patient's hematopoietic system along with malignant cells and ensure engraftment by immunosuppression. The toxicity of these procedures, in terms of very serious side effects, meant that older patients or patients with comorbidities were excludes as possible candidates for HSCT. These patients, however, got the opportunity for treatment with HSCT with the advent of the so-called reduced intensity conditioning (RIC) regimens. This approach relies more on the graft-versus-tumor effect than the conditioning regimen for the reduction of the tumor burden prior to HSCT. Consequently, initial mixed chimerism is observed in the majority of patients undergoing HSCT, with RIC protocols and the need for continuous monitoring of the level of patient's hematopoiesis after HSCT becoming even more pronounced.[6]

The aim of this article is to give a historic view of the methods used for chimerism analysis, describe the currently used gold-standard technique as well as inform about the latest developments and introduction of quantitative PCR technology in this field.

CHIMERISM DETECTION METHODS – A HISTORICAL OVERVIEW

Various different methods have been used throughout the last few decades in order to distinguish the origin of cells in a patient's blood or bone marrow sample after HSCT, and therefore determine the chimerism status of the patient.[7, 8] However, one aspect that all these techniques have in common is the overall procedure which consists of testing the patient and donor for genetic markers, identifying those markers for which the donor and patient differ, and subsequently testing those informative markers in the patient's sample after HSCT. The comparison of sensitivity and applicability of used methods is shown in Table 1.[9]

It is a well-known fact that the discovery of the polymerase chain reaction revolutionized molecular diagnostics in medicine. The identification of the so-called repetitive DNA in the genome happened approximately at the same time, and the combination of these two discoveries led to the establishment of the method for chimerism detection which has been the method of choice for the last three decades.[10]

Table 1. Sensitivity and informativity of methods used for chimerism detection after HSCT

Method	Sensitivity	Informativity
Erythrocyte phenotyping	0.04–3%	Low
Cytogenetics	5–10%	Low
FISH	0.7–5%	High
RFLP	5–10%	High
STR	0.4–5%	Highest
Real-time quantitative PCR	0.1–1%	High

Microsatellite or Short Tandem Repeats (STR) loci belong to the group of highly repetitive DNA, with a sequence comprised of two to six nucleotides repeated a variable number of times. These loci are highly polymorphic, with alleles differing in the number of repeats, and located throughout the entire genome. Although their first application was in the field of forensics, their potential for distinguishing the patient from the donor in the HSCT program was soon recognized. In addition to the fact that informative STR markers can easily be identified for virtually all patient/donor pairs, the method uses PCR amplification of these loci which is a fast, robust and reliable method, requires a small sample quantity and can be performed in the early post HSCT period. Further development of the method involved multiplexing of the PCR. The only disadvantage of the described technique is its relatively low sensitivity of 1-5% due to the fact that there is competition of the same primer for both minor and major cell population. This can be highly improved by performing the analysis on cell subsets; however, the need for cell sorting in such scenario adds to the complexity and is not always feasible.[8, 11]

QUANTITATIVE PCR IN CHIMERISM DETECTION

A decade after the conventional PCR was described, a version of this technique was developed[12] which enabled the measurement of the amount of amplified DNA in real time during amplification, therefore providing a quantitative analysis,. This method was called real-time PCR or quantitative PCR (qPCR). The most widely used approach, the probe-based qPCR, is presented in Figure 1. This approach utilizes fluorescently-labelled target-specific probes. These probes have a fluorescent reporter bound to one end, and a quencher of fluorescence bound to the other end. While the probe is bound to its specific target sequence on the DNA, the reporter and quencher are in close proximity, and the emission of fluorescence is prevented. However, during the elongation step of the PCR cycle, the 5' to 3' exonuclease activity of the Taq polymerase causes the hydrolyzation of the probe, the reporter is released, and emission of fluorescence

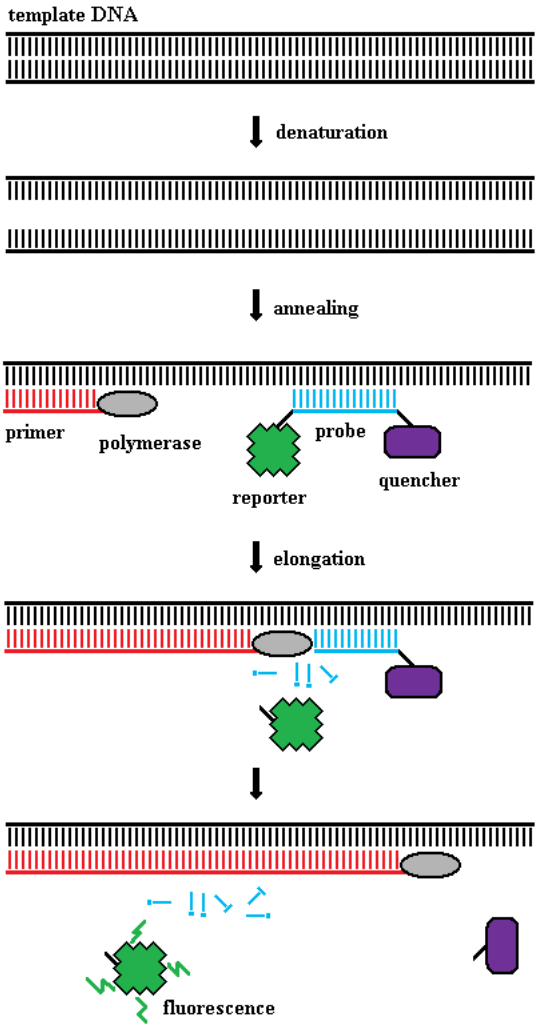

Figure 1. Probe- based qPCR method.

occurs. The amount of the product targeted by the reporter probe is increased in subsequent cycles, which in turn causes a proportional increase of fluorescence.[13] The measurement of fluorescence is performed for each amplification cycle and results in the amplification curve (Figure 2). The comparison of amplification curves of a patient's samples, one taken before and the other after transplantation, provides relative quantification. The comparison requires setting a fixed fluorescence value across the graph, the so-called threshold value. The number of cycles where the fluorescence reaches the threshold value is defined as the Cq value, and this value is inversely proportional to the amount of DNA present in the original sample. The system is calibrated using an internal control, usually by amplification of a housekeeping gene, therefore obtaining a normalized Cq value (ΔCq) ,which is in turn used to calculate the ΔΔCq value (number of additional cycles necessary to reach the threshold value of the post-transplant sample in comparison to the pre-transplant sample). This is finally used to calculate the percentage of the recipient's DNA in the post-transplantation sample (Figure 3).[14]

Although the number of qPCR-based diagnostic procedures increased rapidly since the very beginning, it took almost ten years for the first article to be published describing the new, qPCR-based method for chimerism analysis after HSCT. The sensitivity of the qPCR method of 0.1% represented a notable improvement in comparison to the PCR-STR method and therefore gained the interest of the scientific and medical community, especially in light of the concern that the 1-5% sensitivity limit of the PCR-STR method is insufficient to detect minimal residual disease and predict imminent relapse.[14]

The disadvantages of the initial qPCR assays were that biallelic polymorphisms were analyzed which significantly decreased the applicability/ informativeness of the method in comparison to the PCR-STR technique; and the assays did not reach a reliable quantitative accuracy. The PCR-STR method therefore remained the first choice for chimerism detection throughout the next 15 years.

Recent developments in qPCR-based chimerism assays, however, might change the current practice. Namely, in addition to recently published studies about the qPCR-based chimerism monitoring after HSCT,[15-18] several commercial kits have appeared on the market, utilizing the insertion/deletion polymorphisms, greatly increasing the applicability of the assay by enabling identification of at least one informative marker for >99% of patient/donor pairs. The marker is considered informative for the patient if the patient's sample has a positive reaction while the donor's sample is negative. The opposite rule applies for the markers informative for the donor. Possible combinations and their application are shown in Table 2. The number of informative loci is higher in cases when the donor is an unrelated individual. The sensitivity of the method, which is 20 to 100-fold higher than that of the PCR-STR method, and reduced time necessary to perform the test because no post-PCR procedures are necessary have been incentive enough for many laboratories to switch to qPCR-based methods for the monitoring of the patient's chimerism status after HSCT.

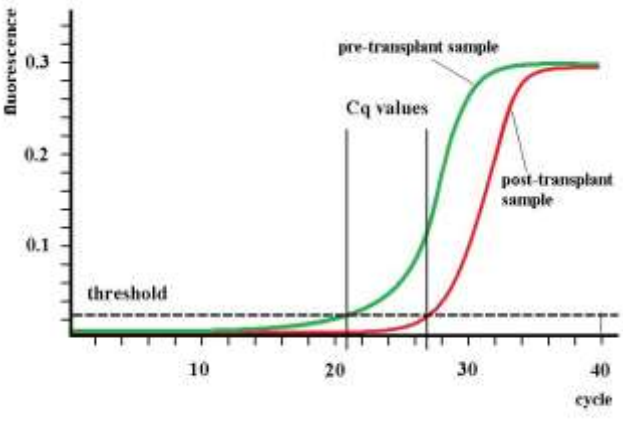

Figure 2. qPCR fluorescence signal curve

Table 2. An example of a qPCR genotyping results

KMR MARKER	PATIENT	DONOR	INFORMATIVE FOR	APPLICATION
KMR013	positive	negative	recipient	patient cells are minor population
KMR028	negative	positive	donor	donor cells are minor population
KMR037	positive	positive	not informative	-
KMR056	negative	negative	not informative	-

CONCLUSION

The high number of different methods and techniques used for the assessment of chimerism status demonstrates how the need for a more accurate, more sensitive and faster method in order to improve the post-transplantation patient care leads to scientific and technological developments. The rapid changes in methodology are another indicator of the importance of chimerism status analysis as it remains one of the major tools for the assessment of graft function, the prediction of disease relapse and making decisions about immunosuppression or medical interventions such as administration of donor lymphocyte infusions.

REFERENCES

1. Szczepanski T, Orfao A, van der Velden VH, San Miguel JF, van Dongen JJ. Minimal residual disease in leukaemia patients. Lancet Oncol. 2001;2:409-417.
2. Socie G, Lawler M, Gluckman E, McCann SR, Brison O. Studies on hemopoietic chimerism following allogeneic bone marrow transplantation in the molecular biology era. Leuk Res. 1995;19(8):497-504.
3. Maloney S, Smith A, Furst DE, Myerson D, Rupert K, Evans PC, Nelson JL. Microchimerism of maternal origin persists into adult life. J Clin Invest. 1999;104:41-47.
4. Socie G, Gluckman E, Carosella E, Brossard Y, Lafon C, Brison O. Search for maternal cells in human umbilical cord blood by polymerase chain reaction amplification of two minisatellite sequences. Blood. 1994;83:340-344.
5. Storb R. Non-myeloablative allogeneic transplantation - state-of-the-art. Pediatr Transplant. 2004;8:12-18.
6. Maris M, Storb R. The transplantation of hematopoietic stem cells after non-myeloablative conditioning: a cellular therapeutic approach to hematologic and genetic diseases. Immunol Res. 2003;28(1):13-24.
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 Transplant. 2005;35(2);107-119.
8. Khan F, Agarwal A, Agrawal S. Significance of chimerism in hematopoietic stem cell transplantation: new variations on an old theme. Bone Marrow Transplant. 2004;34(1):1-12.
9. Baron F, Sandmaier BM. Chimerism and outcomes after allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning. Leukemia. 2006;20:1690-1700.
10. Lion T, Watzinger F, Preuner S, Kreyenberg H, Tilanus M, de Weger R, van Loon J, de Vries L, Cavé H, Acquaviva C, Lawler M, Crampe M, Serra A, Saglio B, Colnaghi F, Biondi A, van Dongen JJ, van der Burg M, Gonzalez M, Alcoceba M, Barbany G, Hermanson M, Roosnek E, Steward C, Harvey J, Frommlet F, Bader P. The EuroChimerism concept for a standardized approach to chimerism analysis after allogeneic stem cell transplantation. Leukemia. 2012;26(8):1821-1828.
11. Kristt D, Stein J, Yaniv I, Klein T. Assessing quantitative chimerism longitudinally: technical considerations, clinical applications and routine feasibility. Bone Marrow Transplant. 2007;39(5):255-268.
12. Higuchi R, Fockler C, Dollinger G, Watson R. Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. Biotechnology. 1993;11:1026-1030.
13. Lee LG, Connell CR, Bloch W. Allelic discrimination by nick-translation PCR with fluorogenic probes. Nucleic Acids Res. 1993;21:3761-3766.
14. Alizadeh M, Bernard M, Danic B, Dauriac C, Birebent B, Lapart C, Lamy T, Le Prise PY, Beauplet A, Bories D, Semana G, Quelvennec E. Quantitative assessment of hematopoietic chimerism after bone marrow transplantation by real-time quantitative polymerase chain reaction. Blood. 2002;99(12):4618-4625.
15. 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.
16. Bach C, Tomova E, Goldmann K, Weisbach V, Roesler W, Mackensen A, Winkler J, Spriewald BM.Monitoring of hematopoietic chimerism by real-time quantitative PCR of micro insertions/deletions in samples with low DNA quantities. Transfus Med Hemother. 2015;42(1):38-45.
17. Ahci M, Stempelmann K, Buttkereit U, Crivello P, Trilling M, Heinold A, Steckel NK, Koldehoff M, Horn PA, Beelen DW, Fleischhauer K. Clinical Utility of Quantitative PCR for Chimerism and Engraftment Monitoring after Allogeneic Stem Cell Transplantation for Hematologic Malignancies. Biol Blood Marrow Transplant. 2017;23(10):1658-1668.
18. Aloisio M, Bortot B, Gandin I, Severini GM, Athanasakis E. A semi-nested real-time PCR method to detect low chimerism percentage in small quantity of hematopoietic stem cell transplant DNA samples. Genome. 2017;60(2):183-192.