CHAPTER 16

Molecular monitoring after HSCT

16.2 Chimerism

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1. Introduction
Monitoring of the ratio of donor- and recipient-derived cells (chimerism) has become an indispensable diagnostic tool in the surveillance of allogeneic hematopoietic stem cell transplant (allo-HSCT) recipients. The increasing employment of reduced intensity conditioning (RIC) regimens and cord blood transplants, which require very careful surveillance of the graft, has further reinforced the clinical importance of chimerism testing. Analysis of chimerism during the post-transplant period permits the assessment of immunological interactions between donor and recipient, providing potentially important information for pre-emptive therapeutic interventions. In addition to enabling the assessment of successful engraftment, analysis of chimerism can provide an early indication of the risk of graft rejection (1–3), and in patients with malignant haematologic disorders, a timely alert of impending relapse (4–6).

2. Technical aspects
Current methodological approaches to chimerism analysis are rather diverse. In the sex-mismatched transplant setting, fluorescence in situ hybridization (FISH) analysis of the X and Y chromosomes in interphase nuclei is regarded as a highly accurate technique for quantitative investigation of chimerism. Technical approaches that can be employed regardless of donor-recipient gender are commonly based on molecular methods including primarily the polymerase chain reaction (PCR). Techniques based on PCR amplification of polymorphic DNA sequences facilitating unequivocal distinction and quantitative assessment of recipient- and donor-derived cells have been the preferred approach to chimerism testing at most centres. Despite the introduction of single nucleotide polymorphism (SNP) and insertion/deletion (Indel) polymorphism analysis by real-time PCR for the investigation of chimerism several years ago, DNA microsatellites [also referred to as short tandem repeats (STRs)] have remained the most commonly used source of polymorphic markers for quantitative assessment of donor/recipient haemopoiesis after allo-HCST. Amplification of STR markers by PCR coupled with fluorescence detection of the donor/recipient alleles using capillary electrophoresis has been a widely used approach to the monitoring of haematopoietic chimerism (7). The main advantages of automated fluorescence-based detection of STR markers over the use of conventional gel electrophoresis include greater precision and easier performance of quantitative analysis, reduced manual handling of PCR products, and higher sensitivity.

2.1 Criteria for the selection of suitable STR markers for chimerism analysis
The accuracy and reliability of quantitative chimerism analysis in post-transplant
samples are greatly affected by factors such as the homozygosity/heterozygosity of STR alleles, shared alleles between donor and recipient, and the positional relationship between alleles. The EuroChimerism (EUC) consortium, an international working party (see below), has therefore established criteria facilitating selection of eligible STR markers for chimerism testing. The EUC group has developed a common descriptive nomenclature for allelic configurations termed the RSD (Recipient-Shared-Donor) code with the aim to facilitate rapid identification of STR markers displaying optimal allele constellations for accurate and reproducible chimerism analysis (8).

2.2 Multiplex versus singleplex PCR assays for chimerism analysis
Multiplex PCR assays are commonly used for initial recipient/donor genotyping to select one or more informative STR markers for the subsequent monitoring of chimerism. Moreover, investigation of post-transplant peripheral blood samples with multiple STR markers may improve the reproducibility and accuracy of quantitative chimerism analysis. The accuracy of quantitative chimerism assays can be increased by testing each sample with more than one marker and calculating mean values (9). Some investigators therefore perform clinical testing of chimerism with commercial multiplex kits facilitating co-amplification of several microsatellite markers in a single PCR reaction (9). However, the indicated advantages may be counterbalanced by the higher cost of consumables and the lower sensitivity resulting from the high number of different fragments co-amplified. Most diagnostic centres therefore rely on the use of singleplex PCR reactions for sensitive assessment and quantitative monitoring of chimerism.

2.3 Chimerism testing within total leukocytes versus analysis of specific cell lineages
PCR-based chimerism assays analysing highly polymorphic STR markers mostly permit the detection of residual autologous cells with a sensitivity limit in the range of 1%. When investigating chimerism in total leukocyte preparations from PB, this level of sensitivity may not be sufficient to allow early assessment of impending complications. It is possible to overcome this problem by investigating chimerism in specific leukocyte subsets of interest isolated by flow-sorting or by immunomagnetic bead separation. Since residual recipient-derived cells can be detected within the individual leukocyte fractions with similar sensitivity, it is possible to identify and monitor minor autologous populations that would escape detection in total PB leukocyte samples. The overall sensitivity of chimerism assays achievable by investigating specifically enriched leukocyte subsets is in a range of 0.1–0.01% (4).
i.e. one to two logs higher than analysis of total leukocyte preparations. Investigation of individual leukocyte fractions therefore not only provides more specific information, but also permits the assessment of impending complications at a significantly higher sensitivity, thus providing a basis for earlier treatment decisions.

2.4 Heterogeneity of current methods and approaches to harmonisation

The methods for STR-PCR analysis of chimerism performed in most diagnostic laboratories are based on various in-house assays. The heterogeneity of technical approaches and the diversity of STR markers used render the comparison of results generated at different centres difficult. Attempts have therefore been made to introduce a commercially available platform for chimerism testing (http://www.biotype.de) or to exploit existing commercial microsatellite kits designed for forensic purposes which, however, are not optimally suited for chimerism testing (8). To address the urgent need for a standardised technology specifically adapted to the requirements of quantitative chimerism analysis, twelve leading centres from ten European countries have established the EuroChimerism consortium to perform a collaborative study supported by the European Commission within the 5th Framework Program. The paramount goal of the consortium was to establish a standardised approach to quantitative chimerism testing, with the aim to facilitate harmonisation of chimerism diagnostics between European centres, and to provide a basis for appropriate quality control. Following extensive analysis of a large set of microsatellite (STR) loci, the EuroChimerism (EUC) marker panel was established. The panel comprises 13 STR markers selected to optimally meet the specific requirements of quantitative chimerism analysis. Based on highly stringent selection criteria (8), the EUC panel provides multiple informative markers in any transplant setting. The EuroChimerism assay provides standardised STR-PCR tests permitting detection of donor- or recipient-derived cells with a sensitivity limit of 0.8–1.6%. Moreover, the assay facilitates accurate and reproducible quantification of donor and recipient haematopoietic cells (Lion et al., submitted). Based on the collaboration of the EUC consortium with an industrial partner (Miltenyi Biotech, Germany) a multiplex PCR kit for the identification of informative STR markers (ChimerXplain™) and a singleplex PCR kit specifically adapted for quantitative chimerism analysis (ChimerXact™) will soon be commercially available, and may serve as a tool for the harmonisation of post-transplant monitoring between diagnostic laboratories. Implementation of the kits in the clinical monitoring of chimerism could help eliminate the problems of heterogeneity in the currently used technical approaches, and could greatly improve the comparability of data and the exchange of information between centres.
3. Detection of imminent leukaemia relapse post-transplant by chimerism analysis

In most instances, surveillance of residual or reappearing leukaemic cells after allo-HSCT is performed by leukaemia- or clone-specific markers. In instances in which specific markers are not available, the exploitation of chimerism testing may be instrumental for the assessment of impending relapse. Investigation of chimerism in total leukocyte fractions from PB was shown to reveal reappearance of autologous cells (mixed chimerism) before the diagnosis of relapse (6), thus providing a basis for timely initiation of appropriate treatment. In a number of instances, however, chimerism testing within total leukocytes may not show any changes indicative of impending relapse (4). Based on its greater sensitivity, investigation of specific leukocyte subsets derived from PB or bone marrow (BM) has a greater potential of revealing informative changes in patients who later experience haematological relapse. Patients with imminent leukaemia relapse can reveal persistence or reappearance of autologous allelic patterns within cell subsets expected to harbour leukaemic cells, if present. These cell populations can be specifically enriched for chimerism testing by targeting the original immunophenotype of the leukaemic clone. The stem cell marker CD34 is commonly expressed by leukaemic cells in combination with lineage-specific markers. For example, tumour cells in B-cell precursor acute lymphocytic leukaemia typically display co-expression of CD34 and CD19. Cell populations expressing these markers can therefore be specifically targeted for the assessment of residual disease by monitoring the presence and the kinetics of recipient chimerism. In some instances, however, the only observation made before haematological relapse is lineage-specific chimerism kinetics suggestive of graft rejection (4). This observation may be attributable to the loss of the graft-versus-leukaemia effect associated with rejection of the allograft.

4. Prediction of graft rejection by the monitoring of chimerism within lymphocyte subsets

Patients who receive reduced-intensity conditioning reveal persisting leukocytes of recipient genotype more commonly than patients who receive myeloablative conditioning. The higher incidence of mixed or recipient chimerism may be attributable both to cells of myeloid and lymphoid lineages (1). In patients who receive T-cell depleted grafts, there is a strong association with the presence of mixed or recipient chimerism within T-cells (CD3+) and NK-cells (CD56+). Detection of mixed chimerism within lymphocyte populations is associated with an increased risk of late rejection (1, 4). In most instances, serial analysis reveals a persistently high or an increasing recipient-specific allelic pattern prior to overt graft rejection (1,
The correlation between the observation of mixed or recipient chimerism and graft rejection was shown to be higher for NK-cells than for T-helper (CD3+/CD4+) or T-suppressor (CD3+/CD8+) cells (1). Patients displaying recipient chimerism in CD56+ cells between days +14 and +35 appear to have an extremely high risk of graft rejection. By contrast, virtually all patients who experience late graft rejection show pure donor genotype within the myeloid (CD14+ and CD15+) cells during the same period (1). Hence, the observation of recipient chimerism within the CD56+ and CD3+ cell subsets is highly predictive for the occurrence of late graft-rejection. These findings underscore the importance of cell subset analysis during post-transplant chimerism testing.

Timely diagnosis of impending graft rejection is crucial for effective therapeutic intervention. In a recent study, the predictive potential of early leukocyte subset-specific chimerism for graft loss has been investigated in children undergoing allo-HSCT for treatment of malignant and non-malignant diseases after reduced-intensity or myeloablative conditioning. Monitoring of lineage-specific chimerism was performed upon first appearance of leukocyte counts amenable to cell sorting. The first chimerism analysis of T- and NK-cells performed at a median of 20 days after HSCT identified three different risk groups which were independent from the conditioning regimen: recipient chimerism (RC) levels in T-cells below 50% indicated a very low risk of rejection (1.4%), while high levels of RC (>90%) both in T- and NK-cells were associated graft loss in the majority of patients (90%) despite therapeutic interventions. Recipient chimerism >50% in T-cells and ≤90% in NK-cells defined an intermediate risk group in which timely immunotherapy frequently prevented rejection. Early analysis of T- and NK-cell chimerism can therefore be instrumental in the risk assessment and therapeutic management of imminent graft rejection (10).

Key points are summarised below.

**Key points**

- Commercial kits for standardised quantitative monitoring of chimerism based on STR PCR analysis will soon be available from Miltenyi Biotech, and may facilitate harmonisation of diagnostics between centres.
- Analysis of chimerism within specific leukocyte lineages can permit earlier assessment of impending complications such as graft rejection or disease relapse.
- Lineage-specific chimerism analysis of T- and NK-cells within the first three weeks post-transplant permits early risk assessment of impending graft rejection.
References


Multiple Choice Questionnaire

To find the correct answer, go to http://www.esh.org/online-training/handbook/

1. Early analysis of cell lineage-specific chimerism after allogeneic stem
cell transplantation permits risk assessment of graft rejection. The following leukocyte lineages are of major importance in this context:

a) B-lymphocytes and plasma cells
b) Granulocytes and monocytes
c) T-lymphocytes and natural killer (NK) cells
d) Donor- and recipient-derived haematopoietic stem cells

2. Impending relapse of leukaemia after allogeneic hHSCT may be revealed by the monitoring of chimerism. Which of the following findings is not suggestive of relapse?

a) Reappearance of recipient cells displaying the immunophenotype of the original leukaemia
b) Increasing recipient chimerism within total leukocytes
c) Chimerism findings indicative of graft rejection
d) Increasing donor chimerism within total leukocytes