The role of chimerism following hematopoietic stem cell transplantation

About the author

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For the last 20 years, he has had an interest in chimerism analysis in transplanted patients, and has led the development of new technologies, including highly sensitive molecular diagnostics for chimerism analysis.

HSCT- a brief introduction

One major medical advancement during the last 60 years has been the introduction of allogeneic hematopoietic stem cells (HSC) from bone marrow (BM), peripheral blood (PB) or cord-blood (CB) as a curative treatment for patients with malignant or non- malignant haematological diseases. In 1957 the first attempts of performing bone marrow transplantation in two patients suffering from a chronic leukaemia were made (Thomas, Lochte et al. 1957). The initial results of these treatments were however very poor with most patients dying in complications directly related to the transplantation. With the detection and increasing understanding of the importance of the HLA system (Human Leucocyte Antigens), results of stem cell transplantations improved markedly. However, even HLA matched transplants suffered graft versus host disease which could only be overcome with continuing improvements in immunosuppressive therapy. (Granot and Storb 2020). Today HSCT is still the only curative treatment for patients with malignant or non-malignant haematological diseases (Gratwohl and Niederwieser 2012, Granot and Storb 2020).

Between the years 1957 and 2016, a total of 1,298,897 HSCT, of which 57.1% autologous transplantations have been performed (Niederwieser, Baldomero et al. 2021). At present, approximately 90 000 patients undergo hematopoietic stem cell transplantation (HSCT) world-wide every year (Niederwieser, Baldomero et al. 2021). The majority of these patients have an underlying malignant disease such as acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), chronic myeloid leukemia in blast phase, Myelodysplastic Syndrome (MDS), multiple myeloma, high-risk lymphomas and Hodgkin's disease. Several non-malignant diseases are also successfully treated using HSCT. Among them are several immunodeficiencies, such as severe combined immunodeficiency (SCID), Wiskott-Aldrich syndrome, common variable immunodeficiency (CVI) (Saba and Flaig 2002).

Although the outcome after HSCT has improved substantially over the years, allogeneic stem cell transplantation is still associated with several potential complications. These complications include toxicity related to the pre-treatment, infections, immune reactions such as graft-versus-host-disease (GVHD) and recurrence of the underlying malignant disease and (Barrett, Horowitz et al. 1989, Horowitz, Gale et al. 1990).

Graft-versus-host-disease (GVHD) is an immune- mediated complication following HSCT and is orchestrated by donor T-cells recognizing allogeneic foreign antigens such as HLA antigens, polymorphic non-HLA antigens or minor antigens (eg HY-peptide) in the recipient. GVHD affects several organs including skin, liver, gastro-intestinal tract as well as the urogenital tract and eyes. Although associated with high morbidity, GVHD is a complication which should not be completely avoided since it has been demonstrated that patients who develop GVHD also tend to develop a graft-versus- leukemia (GVL) (Lulla, Naik et al. 2021).

The GVL effect has been shown to be of major importance, if not the most important mechanism, for eradication of remaining leukemic cells in the patient (Dickinson, Norden et al. 2017, Orti, Barba et al. 2017).

Another major complication after HSCT, is the incomplete eradication of the remaining leukemic cells with potential recurrence of the leukemia in the patient. Recurrence of the underlying malignant disease (relapse) is the most frequent cause of treatment failure in patients undergoing HSCT for leukaemia (Horowitz, Gale et al. 1990, Marmont, Horowitz et al. 1991, van Besien 2013, Dickinson, Norden et al. 2017). For instance, approximately 40% of post-SCT AML patients will relapse and face a poor prognosis with a 2-year survival of less than 20% (Tsirigotis, Byrne et al. 2016). Possible treatment options for relapsed patients include tapering of immunosuppression, additional chemotherapy followed by donor lymphocyte infusion (DLI) or second allogeneic- SCT (Kolb and Bender-Gotze 1990, Orti, Barba et al. 2017). Since the complete eradication of the malignant cells is difficult to measure, complete remission (CR) has been used for defining successfully treated patients. Complete remission in acute leukemias is defined as <5% bone marrow blasts, no extramedullary disease and the absence of any aberrant cells with leukemia immunophenotype by flow cytometry during early assessment with persistent neutropenia and thrombocytopenia (Cheson, Bennett et al. 2003). The presence of very small numbers of remaining malignant cells in blood or bone marrow has been termed minimal residual disease (MRD) (Zhou, Othus et al. 2016).

The monitoring for the presence of minimal residual disease has become an important strategy at many centers to identify patients at risk for relapse (Walter, Gyurkocza et al. 2015, Tsirigotis, Byrne et al. 2016). Therefore, in addition to treatment strategies for preventing leukemic relapse as described above, development of several techniques for measuring mixed chimerism, MRD and detection of relapse have been of major importance for the outcome in these patients. These methods include multicolour flow cytometry, different techniques for molecular monitoring of MRD markers and chimerism analysis (Tsirigotis, Byrne et al. 2016).

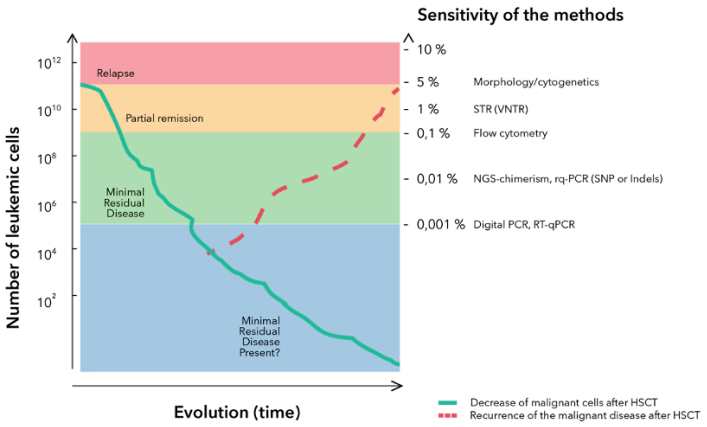

Fig. 1 This figure depicts a theoretical evolution of the malignant cells after HSCT. If the HSCT is curative (green line), the number of malignant cells is decreasing below detection with current diagnostic techniques. In case of increasing MRD (red line) the number of malignant cells may increase from sometimes know levels to a clinical relapse. This increase is in many cases associated with increasing mixed chimerism (IMC) and can be detected by the methods indicated in the figure. This detection is restricted by the sensitivity that the different methods exhibit.

Chimerism analysis- a brief background

The expression chimera derives from the Greek mythology and was first described by Homeros in the Iliad as a fire-breathing creature in minor Asia composed of the parts of multiple animals. Chimerism in medicine is defined as the presence of cells, in humans or animals, that originate from another individual and therefore differ genetically from the cells of the host individual. This situation can occur naturally during pregnancy, where fetal cells circulate within the maternal blood, in dizygotic twin pregnancies with separate placentas or after transplantation (Bianchi, Khosrotehrani et al. 2021, Rosner, Kolbe et al. 2021). The fact that cells from two (or more) genetic separate individuals can co-exist side-by-side within one body has led to development of new methods sensitive enough to detect even very small amounts of foreign DNA and RNA to discriminate the amount of the two genetic individuals within the organism (Alizadeh, Bernard et al. 2002, Pettersson, Vezzi et al. 2021).

Mainly the clinical need for monitoring chimerism after HSCT has led to the development of several new molecular techniques. The general principle of these methods is the use of genetic differences or polymorphisms that exists between individuals. The most common polymorphic sequences include short tandem repeats (STR), single nucleotide polymorphisms (SNP) and Insertions-deletions (Indels). PCR amplification of these polymorphic DNA sequences has been the preferred method due to high sensitivity of detecting the lower cell fraction and the possibility of quantitative assessment of recipient and donor cells (Gineikiene, Stoskus et al. 2009).

Different terms are used to describe the chimerism status after HSCT (Antin 2001, Antin, Childs et al. 2001). Complete or donor chimerism (DC) means that only donor cells are detected whereas mixed chimerism (MC) describes the presence of both recipient and donor cells in the sample analyzed (e.g blood or bone marrow). MC can further be subdivided into stable (SMC), decreasing (DMC) or increasing MC (IMC) depending on the kinetics of the recipient levels. These terms are summarized in Table 1. The definition of chimerism status is however complicated by the sensitivity of the method used. While some methods have a sensitivity of 3-5% (STR), others can detect recipient levels down to 0,1-0,01% (digital PCR, rq-PCR and NGS-based techniques). A recipient level of 1 % can, therefore, be classified as DC with one method but MC with another. Today, most studies refer to DC if recipient levels are not detected above 1% using total DNA as reference material (Svenberg, Mattsson et al. 2009, Rettinger, Willasch et al. 2011).

Term	Definition
Complete Chimerism (CC)	Only donor DNA is detected; 100% donor
Mixed Chimerism (MC)	Both donor and recipient DNA are detected
Stable	Both donor and recipient DNA detected; % recipient DNA not changing significantly compared to previously tested timepoint/ sample
Increasing	% recipient DNA is increasing compared to previously tested timepoint/sample
Decreasing	% recipient DNA is decreasing compared to previously tested timepoint/sample
Split Chimerism	Complete chimerism in one or more cell subsets with mixed chimerism or 100% recipient in other cell subsets
Microchimerism	Less than 1% recipient DNA detected
Autologous Recovery	Only recipient DNA is detected; 0% donor

Tab. 1 Post-transplant chimerism terminology

One further improvement of the sensitivity of chimerism analysis following HSCT has been the implementation of lineage-specific chimerism in blood and bone marrow. Already in 1995, Socie et al suggested that in order to understand the dynamics of engraftment following HSCT, specific cell subsets should be investigated (Socie, Lawler et al. 1995). Since then, several studies have shown that lineage-specific analysis or split chimerism is important when investigating the kinetics of engraftment after HSCT and monitoring for MRD and relapse (Roux, Helg et al. 1992, Roux, Abdi et al. 1993, Roux, Helg et al. 1996, Zetterquist, Mattsson et al. 2000, Mattsson, Uzunel et al. 2001). Lineage-specific chimerism analysis has thus helped to further increase the sensitivity of the tests.

Chimerism analysis- a tool for clinical decisions

To maximize the potential of the chimerism assay in the clinic, several factors should be taken into consideration. For instance, sampling time, monitoring technique and sample material all influence the power of the assay. All these variables improve the clinical significance if used adequately.

Sampling time

To understand the distribution and kinetics of recipient and donor cells in the patient, regular sampling at different time points is needed. Donor cells usually emerge in low amounts in blood samples as early as 7 days after HSCT and full donor engraftment can in many cases be detected 2-3 weeks post-HSCT (Dubovsky, Daxberger et al. 1999) (O'Reilly, Meyer et al. 1993). The first chimerism assessment is usually done 2-3 weeks after HSCT to verify engraftment.

There were early recommendations that chimerism analysis should be done weekly for 100 days and every month thereafter in patients with leukemia but these programs may have been adjusted to local protocols (Bader, Niethammer et al. 2005). In any case, since most relapses occur during the first year after HSCT, frequent chimerism assessment is important during this time period. Although, these recommendations mean higher costs, the approach has been successful for early prediction of relapse (Haugaard, Kofoed et al. 2020). In contrast, less intensive sampling may be executed in non-malignant diseases where higher amounts of MC can be tolerated.

Monitoring technique

The analysis with capillary electrophoresis (STR) is the most widely used method for chimerism assessment and is currently considered gold-standard. Although this may partly be explained by the early development of the method and adapted in-house protocols over time, the need of relatively few markers to discriminate recipient and donor pairs and the accurate quantification of the method are factors that have made the method useful in many clinical laboratories. However, due to a limited sensitivity of the assay of 3-5%, the time between mixed chimerism detection and relapse may be too short for immune therapy to have an effect (Zeiser, Spyridonidis et al. 2005). The advantage of a more sensitive technique, such as real-time PCR or dd-PCR, is the high sensitivity, which can allow the clinician to follow the dynamics of chimerism already at very low recipient levels (<1 %). Some studies indicate that real-time PCR is superior to the STR method for detection of relapse after HSCT (Jimenez-Velasco, Barrios et al. 2005) (Koldehoff, Steckel et al. 2006).

The drawback with this method is its inherent poor precision at increasing amounts of MC. Therefore, some laboratories are running several methods in parallel.

Recent development of NGS-based chimerism assays exhibiting all the advantages of STR and real-time PCR for chimerism without the previously described drawbacks may prove to be the technique for chimerism analysis of the future. Slightly increased turn-around- times for samples in the laboratory should be related to the ability of the NGS-based assay of higher sample through-put. Taken together, laboratories with the necessary infrastructure for NGS sequencing may well profit from this emerging technology for chimerism analysis.

Sample material

Since most of the malignant hematological diseases are primarily located in the bone marrow, chimerism analysis in the bone marrow and in the peripheral blood has been studied. The literature comparing chimerism analysis using peripheral blood or bone marrow does not clearly favor any of these sample materials. In one study for instance, chimerism analysis was compared in patients who relapsed with those in stable remission using a sensitive quantitative PCR method of both PB and BM (Qin, Li et al. 2012). This study demonstrated an increased recipient chimerism in BM samples with a defined threshold of ≥0.5% observed in 90% of patients before relapse. The study also reported that in paired BM and PB samples collected at time of relapse, all BM samples had significantly higher recipient DNA % than PB samples. However, detection of MC in BM earlier and at a higher frequency than in PB does not necessarily imply higher diagnostic utility on BM chimerism. It is also unclear whether early changes in chimerism detected only in BM reflect normal dynamic changes of a regenerating BM. Only frequent sampling may answer this question and here blood may be more convenient. In a recent study of patients that showed CC by STR, qPCR revealed that 59% had 0.1–1% recipient DNA in BM while only 7% presented 0.1–1% recipient DNA in PB (Navarro-Bailon, Carbonell et al. 2020). Of the latter group one of four patients relapsed suggesting that the detection of recipient DNA in PB is much less frequent than in BM but may have superior clinical utility in early detection of disease relapse. In a prospective study of twenty patients with high-risk AML chimerism analysis was evaluated with respect to performance of serial monitoring using qPCR chimerism analysis in BM vs PB for the prediction of relapse (Gambacorta, Parolini et al. 2020). Paired BM and PB samples showed moderate correlation with, in general, higher recipient DNA % in BM compared to PB, regardless of relapse. In this study, the most predictive results of relapse were achieved in both PB and BM when MC was exceeding the threshold values of 0.13% for PB and 0.24% for BM.

Here too, the authors suggested that more frequent sampling may improve the predictive values of chimerism analysis which is feasible with the less invasive PB compared to BM.

Significant differences in the ability to interpret chimerism monitoring also relies on whether total DNA or cellular subsets are studied. For instance, chimerism analysis in specific cellular subsets offers several advantages as compared to total DNA analysis from unsorted PB or BM samples. For instance, mixed chimerism in T-cells can predict GVHD and graft failure, (Childs, Clave et al. 1999) (Mattsson, Uzunel et al. 2001) while mixed chimerism in disease affected cellular subsets, e.g CD19+ cells in B-ALL or CD34+ cells in AML, is usually correlated with high risk of relapse (Bornhauser, Oelschlaegel et al. 2009) (Mattsson, Uzunel et al. 2001) (Zetterquist, Mattsson et al. 2000) (Lindahl, Vonlanthen et al. 2022). Since analysis of chimerism in total DNA may be hampered by the contamination by DNA from other cells, such as granulocytes, several laboratories have introduced cell sorting to improve the power of chimerism analysis (Mattsson, Uzunel et al. 2001).

Taken together, in addition to BM collection being more invasive than PB, there appears to date to be no conclusive evidence to support a superior prognostic performance of chimerism studies on BM as compared to PB. Moreover, monitoring for MC using enriched cells relevant for the disease, further strengthens the power of the assay and could be suitable for MRD monitoring.

The concept of microchimerism

A term frequently used in the literature, when studying chimerism post-HSCT, is microchimerism. Microchimerism is defined as MC <1 % in the sample of interest and may be of importance when chimerism is analysed in the context of MRD and relapse. Progression of MC to CDC generally occurs in the first 1–2 months post-HSCT and association with relapse risk is mainly found for patients with persistent IMC (Bader, Niethammer et al. 2005), (Bader, Holle et al. 1997), (Konuma, Kato et al. 2016) (Lindahl et al, BMT In press, 2022). When studying microchimerism, the decline of recipient DNA at levels below 1% appears to be a gradual process, occurring over the course of 3–6 months post-HSCT (Ahci, Stempelmann et al. 2017), (Sellmann, Rabe et al. 2018), (Elkaim, Picard et al. 2014), (Haugaard, Madsen et al. 2019). These findings posed the question if the kinetics of achieving CDC below 1% could be associated with outcome. A recent study by Lindahl et al (Lindahl, Vonlanthen et al. 2022) showed that early complete chimerism post-HSCT in patients with AML, correlated inversely with relapse during the observation time. In this study, complete chimerism was defined as host DNA

<0.1% in CD33+ enriched cells within the first 60 days after HSCT. The study suggests that achievement of CDC assessed by sensitive methods detecting microchimerism early after HSCT appears to be useful for risk stratification in patients transplanted for AML. Other studies compared the achievement of CDC as well as the speed of achieving CDC in relation to relapse risk. Wiedemann et al. found improved 2-year OS and EFS and decreased CIR for patients achieving maximal level of donor chimerism after or before day 50 post-HSCT (Wiedemann, Klyuchnikov et al. 2010). In contrast, Elkaim et al. studied whether achieving versus not achieving CDC predicted relapse and found no evidence to support this (Elkaim, Picard et al. 2014). Taken together, although there are studies supporting the importance and time frame of reaching CDC, additional studies are needed to strengthen these findings.

Summary

Monitoring for mixed chimerism in patients after HSCT has in several studies been shown to be of clinical importance. The main purpose of the assay is to verify engraftment and subsequently monitor for the presence of MRD and potentially early detection of possible relapse. Therefore, some important points could be emphasized. Firstly, it is important to use an assay that offers high precision and sufficient sensitivity since it is important to monitor for increased amounts of host DNA even at low levels. Relevant technical infrastructure is therefore a prerequisite for implementing the assays. Secondly, frequent sampling is an advantage to monitor the dynamics of MC especially if the center is interested in microchimerism as a diagnostic tool for MRD detection. To minimize the inconvenience for the patient of frequent bone-marrow sampling, peripheral blood may be an acceptable sample source for frequent chimerism monitoring.

Finally, chimerism analysis in DNA from cell-sorted samples may be preferred over total DNA to reduce noise from DNA of non-relevant blood cells. This may be a bit more laborious but excellent automated solutions are available on the market today. Using the best available monitoring techniques available today, minimizes the risk of missing out on invaluable information needed to identify MRD and prevent relapse.

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