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Chapter 4

Monitoring of Chimerism Following Hematopoietic Stem Cell Transplantation

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Abstract

One of the most important events in the posthematopoietic stem cell transplantation is the immune system reconstitution—a process characterized by a considerable dynamic. During this period, patients are exposed to different life threatening complications. In this chapter, we consider chimerism levels in relation to the conditioning regimens and disease type. Furthermore, the predictive role of chimerism analysis as an important method in monitoring the early diagnosis of graft versus host disease (GVHD), minimal residual disease (MRD), graft failure or rejection, and disease relapse has been discussed.

Keywords: chimerism, HSCT, STR, conditioning

1. Introduction

In March 1969, Prof. Thomas and his team performed the first transplantation of hematopoietic stem cells (HSCs). Since then, the transplantation of bone marrow or peripheral stem cells has become a routine method for treatment of a number of malignant and nonmalignant hematologic diseases [1–6]. Allogeneic stem cell transplantation is effective in restoring normal hematopoiesis and is a preferred therapeutic method for malignant diseases of the blood, due to its graft-versus-leukemia (GVL) effect. This effect is mainly due to donor T cells that exhibit immunoreactivity against the minor histocompatibility antigens (mHags) of the recipient or epitopes specific for leukemic cells. The immune system recovery after allogeneic hematopoietic stem cells transplantation is of crucial importance during the postoperative period. This process lasts for months to years and depends mainly on the ability of the donor hematopoietic stem cells to take over the recipient cells.

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The determination of the genetic origin of hematopoiesis is referred to as chimerism analysis. The term "chimera" was introduced in medicine by Anderson and coworkers [7] in 1951, and in transplantology—by Ford [8] in 1956. It is used for people who have cell populations from different individuals of the same or a different type that arise occasionally during pregnancy or blood transfusions or are targeted—by transplantation of tissues, organs, and cells. The term chimerism refers to the coexistence of cells from two different organisms in a body.

Each stage of the transplant process potentially exposes the patient to complications and life-threatening events; this is largely due to a lack of understanding of the mechanisms of engraftment, as well as the genetic differences that exist between donor and recipient. Additional factor for consideration is the sensitivity of the methods used. The most widely used one—PCR-STR, is thought to have 1–5% sensitivity. Factors, such as type and stage of disease, patient age, donor type, HLA compatibility, number of transplanted $CD34^+$ cells, graft T cell depletion, and many others, influence the postSCT immune reconstitution, as well as all subsequent complications.

The recovery of hematopoiesis depends mainly on the possibility of the donor hematopoietic stem cells to generate progenitor cells and repopulate the bone marrow niches. It creates a dynamic donor-recipient chimera, the exploration of which—qualitatively or quantitatively, has become an important component of the posttransplant monitoring of the patients. Chimerism is an important indicator for relapse, graft rejection, minimal residual disease (MRD), and graft versus host disease (GVHD). The presence of persistent or emerging recipient cells could mean surviving leukemic cells that could lead to a recurrence of a malignant branch by inhibiting immunocompetent donor cells. Therefore, the accurate assessment of chimerism in the patient's blood or bone marrow provides important information about the engraftment process and aids in providing a more adequate treatment to the recipient. Initially, full donor hematopoiesis was thought to be essential for the survival of the graft after allogeneic stem cell transplantation [9], but in recent years it became clear that posttransplantation chimerism is a dynamic process, and patients with full donor chimerism (FDC) in a posttransplant period may subsequently develop mixed chimerism and vice versa—patients with mixed can develop complete chimerism.

2. Chimerism and different therapeutic strategies

Patients undergoing allogeneic SCT are given chemotherapy alone or in combination with radiotherapy prior to reinfusion of blood or a bone marrow graft. In autologous transplantations, the conditioning aims to eradicate tumor cells, whereas in allogeneic transplantations, it aims to immunosuppress the recipient (for the purposes of preventing graft rejection), provide better control of possible GVHD, eradicate tumor cells (which correlates mainly with the intensity of the regimen used), and allow immune reconstitution. There are some exceptions, like recipients with severe aplastic anemia or those with severe combined immunodeficiency who do not require full immune system eradication.

Treatment with chemotherapy or radiation therapy results in severe depletion of all hematopoietic cells of the immune system. Both alkylating chemotherapeutic agents and irradiation target highly proliferative cells [10–12], including developing and naïve lymphocytes, making

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them particularly depleted following treatment [13]. Conditioning regimens cause neutropenia, which are likely to last up to approximately 30 days depending on the source of stem cells [14]. Delayed recovery following immunodepletion is associated with a high degree of morbidity and mortality [13, 15, 16]. Lymphoid recovery is critically dependent on primary immune system organs—the younger the patient, the faster the recovery of $CD4^+$ T cells, B cells, and NK cells [17–20]. Recovery of NK cells after HSCT depends on an expansion of the cytokine-producing $CD56^{bright}$ NK cell subset. Initial recovery of the T cell compartment relies on the peripheral expansion of memory T cells, driven by cytokines and the presence of alloreactive antigens [14]. Interestingly, $CD8^+$ T cells recover at similar rates in young and aged patients, which could be due to extrathymic clonal expansion [20–23]. The recovery of B cells takes longest time—up to 2 years and is preceded by expansion of transitional $CD19^+CD24^{++}CD38^{++}$ B cells [14]. Furthermore, immunodepletion from pretransplant conditioning causes enhanced senescence of the hematopoietic system coupled with an upregulation in the cyclindependent kinase inhibitors $p19^{Arf}$ and $p16^{ink4A}$ mimicking some of the age-related effects [24, 25].

With the development of less intensive conditioning regimens, the frontiers of hematopoietic stem cell transplantations have become even broader covering more diseases and more people becoming eligible for this treatment. The myeloablative regimens (MAs) are designed to fully eradicate the host immune system and facilitate the engraftment of donor cells. In contrast, reduced-intensity conditioning (RIC) and nonmyeloablative (NMA) regimens aim to suppress the immune system in view of preventing donor cell rejection rather than ablate it. Protocols using lower intensity conditioning regimens have been developed to treat hematological disorders in patients with medical comorbidities or elderly people who are not considered appropriate candidates for more intense and toxic conditioning. Thus, the duration of cytopenia is shorter and initial mixed donor chimerism (DC) is more likely to occur in those patients.

In 2009, Bacigalupo et al. published a report proposing a categorization of conditioning regimens into three categories—myeloablative conditioning, reduced-intensity conditioning, and nonmyeloablative conditioning. These categories were distinguished on the basis of cytopenia duration and the need of stem cell support [26]. The terminology reflects the early regimen-related toxicity to the host bone marrow cells, but not the biological effect of the transplant.

- *Myeloablative conditioning regimens*

The MA protocols include administration of high doses of total body irradiation (TBI) and/or alkylating agents, which do not allow autologous hematologic recovery and should benefit a faster donor cell engraftment. Since it is unlikely to fully eradicate a person's immune system, the term myeloablation should be considered as an operational definition, indicating a regimen usually causing an irreversible pancytopenia [26]. The myeloablative conditioning of patients results in higher transplant-related mortality (TRM) and higher frequency of GVHD, compared to other protocols.

- *Reduced-intensity conditioning regimens*

RIC regimens are considered an intermediate category of regimens, which cannot be classified as either NMA or MA—they cause cytopenia that may be prolonged, but the dose of

alkylating agents or TBI is reduced by at least 30%, as compared to MA, and they do require stem cell support [26].

- *Nonmyeloablative conditioning regimens*

NMAs typically cause minimal cytopenia—they do not require stem cell support, but are immunosuppressive to the extent that, when followed by G-CSF mobilized peripheral blood stem cells (PBSC), they usually result in full donor cell engraftment. According to Bacigalupo et al., the NMA regimens are more "immune-ablative" than myelo-ablative [26]. The NMA conditioning results in lower TRM, as compared to MA but requires a larger amount of donor cells to facilitate a full replacement of the recipient's hematopoiesis [27, 28]. Acute GVHD after NMA is delayed and may develop after day +100, at a time when chronic GVHD is usually diagnosed after MA regimen. GVHD remains a significant cause of morbidity and mortality after the application of both MA and NMA [29].

Many studies address the importance of chimerism monitoring after allo-SCT, but the data are controversial [30–36]. Moreover, chimerism monitoring in patients with MA conditioning is considered to be less informative and unnecessary, since the aggressive pretransplant preparation is thought to result in irreversible pancytopenia and achievement of stable full donor chimerism [30, 37, 38]. The chimerism status seems to be even more complicated as new data show the vague borders between the postSCT effects of the different conditioning protocols [31, 39, 40].

The aggressive pretransplant therapy is more effective against the disease, but its use is limited to younger and healthier patients who are able to tolerate concomitant toxicity. MA conditioning aims to facilitate normal hematopoietic reconstitution with FDC by day +30, after allo-SCT. In unmanipulated grafts, chimerism analysis shows mostly FDC, as its early establishment may be an indication of GVHD. Mixed chimerism is more likely to be observed in patients who had received T cell-depleted graft. T cell depletion is used to reduce the frequency of GVHD—Mickelson et al. showed a significantly increased risk of developing acute or chronic GVHD in patients who reached levels of donor chimerism in T cells >90% (HR = 1.92, P = 0.08 and HR = 2.26, and P = 0.07, respectively) [31]. Moreover, speed and extent of donor chimerism have been shown to be influenced by additional factors including primary diagnosis and previous treatment [37].

2.1. Investigation of chimerism in myeloablative HSCT

From a historical point of view, ablative chemotherapy and total body irradiation were accepted as a treatment for elimination of malignant cells and targeting immunosuppression with view of facilitating donor cell engraftment. High dosage protocols aiming to achieve antileukemic effect are still in use for patients with aggressive malignant diseases. The main treatment includes cyclophosphamide and TBI or busulfan (BU), and more often a combination of busulfan and fludarabine at dosages eliminating all myeloid cells [41]. The aggressive pretransplant conditioning is very effective for the primary disease, but it is limited to younger patients who are able to tolerate treatment-related toxicity. Myeloablative conditioning schemes could facilitate reconstitution of hematopoiesis with a complete lymphocyte chi-

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merism at day 30 after HSCT. In such type of HSCT, cases of GVHD are more frequent, while relapses have been rarely observed. Chimerism analysis shows complete donor chimera [37]. In 50–100% of the patients transplanted with T cell-depleted bone marrow grafts, mixed chimera have been observed. In these transplant procedures very often donor-lymphocyte infusion is performed, especially in CML patients.

2.2. Investigation of chimerism in nonmyeloablative/reduced-intensity HSCT

One of the main factors allowing increase in the number of HSCT is the development of nonmyeloablative conditioning and reduced-intensity conditioning regimens. The introduction of these protocols is related to the lower treatment-related mortality, allowing the application of HSCT as a treatment for elderly people and patients in a severe clinical condition. Another substantial advantage of these protocols is the better immune reconstitution due to the lower degree of thymus injury, despite the fact that studies showed similar outcomes for HSCT. This allows better regeneration of naïve T cells and proliferation of immune-competent cells of the recipient, which have survived after conditioning. The majority of RIC protocols are based on a combination of purine analogs, usually fludarabine with alkylating agents, such as busulfan or cyclophosphamide, and represent a different intensity of myelosuppression [42–44]. Another approach is to use low doses of TBI, alone or in combination with fludarabine [45]. This approach relies on the GVL effect and is associated with minimal toxicity. Several studies have shown the association between patient conditioning and posttransplant chimerism levels [46, 47].

Unlike HSCT with myeloablative conditioning, nonmyeloablative conditioning is related to a mixed chimerism [6]. Additionally, the probability to develop aGVHD is lower, while probability to develop cGVHD is comparable for both conditioning protocols. The immunobiology of allo-SCT after NMA conditioning differs from that after MA mainly by the release of less inflammatory cytokines. Tissue damage caused by myeloablation is translated into proinflammatory cytokines that are supposed to provide a milieu for the development of GVHD. As a result, aGVHD after NMA is delayed and may develop after day +100, remaining a significant cause of morbidity and mortality in NMA SCT patients. Mixed chimerism does not mean grim prognosis but could be associated with increased risk of relapses.

Relatively decreased aGVHD after RIC HSCT could be explained by a combination of pretransplant conditioning and initial mixed chimerism that could contribute to the development of tolerance [29, 45]. Some patients demonstrate late development of aGVHD that coincides with the transition from mixed to complete donor T cell chimera [46]. In patients who do not develop GVHD, donor lymphocyte infusion could be performed in order to achieve complete chimera and GVL effect. Therefore, in HSCT with nonmyeloablative conditioning, it is very important to monitor chimerism level in order to assess correctly the need of donor lymphocyte infusion and the effect of this treatment [37]. Lower levels of T and NK cells in the first 30 days following HSCT are associated with increased risk of graft rejection. Granulocyte and monocyte chimerism levels have limited relevance for graft rejection [46]. In contrast, high levels of donor T cells are predictive for development of aGVHD grade 2–4 [31, 46, 48]. Additionally, levels above 90% donor T and/or NK cells are associated with antitumour response and disease-free survival [46,

49]. Similar results have been reported by Mohty et al. (n = 102) [48]. In contrast, some studies do not reveal an association between the level of donor T lymphocytes and the development of GVHD [50, 51]. In their study, Lim et al. showed that rapid conversion to complete donor chimera is associated with decreased overall survival and increased TRM when compared with persistence of mixed chimerism. No association with relapse frequency was observed [52]. Mixed chimera is related to better survival—this observation could be explained by the persisting recipient cells, associated with resistance to infections and overall survival [52, 53]. Patients with low absolute count of dendritic cells, one month following HSCT, have a higher TRM (60% versus 12%, $P < 0.02$) and decreased overall survival (15% versus 45%, $P < 0.002$) [54]. There is also an association between decreased $CD16^+$ dendritic cells and increased infection-related mortality (50% versus 0%, $P < 0.05$). The possibility that the infection itself is likely to decrease the number of these cells cannot be excluded [54].

2.3. Chimerism following donor lymphocyte infusion (DLI)

Donor lymphocyte infusion is a new therapeutic strategy, applied mainly in cases of nonmyeloablative, T cell-depleted myeloablative HSCT, or haploidentical transplantations due to its GVL effect. These patients have delayed immune reconstitution, increasing the risk of disease relapse. Application of standard DLI is limited due to GVHD and aplasia. New protocols using G-CSF-stimulated blood progenitor cells, allodepleted donor T cells and mHag-specific $CD8^+$ cytotoxic T lymphocytes have been currently developed allowing wider application of this type of therapy. A limitation of the latter is estimating the right time for DLI. Lymphocyte infusions in the early relapses are associated with favorable outcome [55, 56]. Therefore, chimerism should be monitored regularly in order to detect recipient-specific hematopoiesis [6, 57, 58]. Infused lymphocytes are sensitized by surface antigens that are associated with leukemia or polymorphic minor histocompatibility antigens, which are expressed by the leukemic cells. This induces their transformation into cytotoxic lymphocytes killing cancer cells. DLI is most effective in patients with CML (approximately 75%), which could be explained by the antigen-presenting cells found in malignant clones. This therapy is not effective in patients with AML or multiple myeloma. The development of new therapeutic protocols has decreased aplasia and GVHD.

3. Chimerism in nonmalignant diseases

Currently, a major part of inherited or acquired nonmalignant diseases, such as thalassemia, aplastic anemia, and immune deficiencies, can be successfully treated by HSCT. The aim of such type of transplantation is to achieve stable engraftment, enabling hematopoiesis, enzyme activity, or immune competence. Therefore, establishment of complete donor chimera is not necessary and the recipient can be conditioned by nonmyeloablative therapy. This results more often in the establishment of mixed chimera, increased risk of graft rejection, and decreased risk of GVHD. On the other hand, rapid development of complete chimera in NK and T cells is very important for the successful engraftment, although it increases the frequency of acute GVHD [59–61].

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4. Chimerism in malignant diseases

Monitoring MRD and chimerism levels in patients with malignant diseases is very important. Allogeneic HSCT is an effective therapy in both low- and high-risk CLL. The analysis of 44 high-risk patients with 17p deletion and RIC transplantation showed a four-year cumulative frequency of disease progression of 34% [62]. Establishment of T cell mixed chimera 90 days following transplantation and chemorefractory disease are associated with increased risk of disease progression. Establishment of complete donor chimera and lack of MRD are associated with prolonged DFS. Limited data on the relapse outcome in CLL are available and some patients have positive response to DLL treatment and immunosuppression withdrawal. A better response has been observed in patients with 100% donor T cells. Many studies have shown that conversion from mixed to complete donor chimera predicts development of GVHD [46, 62–64].

In patients with acute leukemia (AML and ALL), mixed chimera may be associated with increased risk of graft rejection. A greater risk has been demonstrated in patients with high levels of residual cells and/or rapid increase of recipient cells [2, 4, 5]. Monitoring of the level of expression of WT1 and the kinetics of nonseparated CD34 chimerism allows early detection of relapse in MDS and AML patients. In patients with stable $CD34^+$ levels and WT1 expression under predefined cut off, relapse could be excluded within the following 28 days [34, 65, 66].

Establishment of mixed chimera can be associated with many factors such as conditioning intensity, T cell-depleted grafts, CD34 cell dosage, techniques used for monitoring, and primary disease. Due to the dynamics in chimerism development, chimerism should be monitored at shorter intervals, especially in the first 1–2 months following transplantation when the risk of rejection is higher. In the early posttransplant period, the persistent mixed chimera could indicate graft rejection or early relapse. On the other hand, the increased number of recipient cells at a later stage after transplantation is associated with a relapse or late rejection.

Due to its low sensitivity of about 1%, chimerism analysis could be used as a prognostic rather than an indirect indicator for MRD. With the introduction of RQ-PCR technique, the sensitivity of chimerism monitoring has improved to 0.1%, resulting in improvement of its prognostic value for MRD detection.

5. Chimerism analysis: technical considerations

Expanding the curability of hematological diseases with stem cell transplantation, as well as introducing new conditioning protocols allows more patients to be eligible for this treatment. This raises the need for better posttransplant chimerism monitoring, since the different quantitative or semiquantitative methods have their advantages and disadvantages. While the significance of the absolute value is still under discussion, the relative changes in engraftment

kinetics are a reliable sign for acceptance or rejection of the graft. This, in its turn, requires a determination of the factors intrinsic for the platform, which may influence the observed changes in chimerism result [67, 68]. Some of the most important characteristics of the methods for postSCT chimerism monitoring are their sensitivity, precision, and reproducibility [69–71].

Investigating microsatellites (STR) and minisatellites (VNTR) is considered the most sensitive and informative technique to study the levels of chimerism after HSCT. Micro (2–5 bp) and minisatellites (9–80 bp) are tandemly repeated blocks of noncoding DNA that are widespread throughout the human genome. The repeat-pattern of these blocks varies greatly among individuals in a population. There is a large number of STR (short tandem repeats) systems that have been mapped throughout the human genome, and they are found on almost every chromosome (**Table 1**).

Chromosome	STR markers	RFLP-based VNTR markers	Other PCR-based markers
1	F13B, RENA4, D1S1171, D1S1627, D1S1656, D1S1677, D1S2142, and D1GATA113	D1S7 and D1S339	D1S80
2	APOB, TPOX, D2S410, D2S441, D2S436, D2S1242, D2S1338, D2S1360, D2S1772, and D2S1776	D2S44	ApoB
3	ACPP, D3S1349, D3S1352, D3S1358, D3S1359, D3S1545, D3S1744, D3S3053, and D3S4529		
4	FABP, FGA (FIBRA), GABARB15, D4S2364, D4S2366, D4S2368, and D4S2408	D4S139	GC (PM) and GYPA (PM)
5	CSF1PO, D5S373, D5S815, D5S818, and D5S2500	D5S110	
6	F13A1, FOLP23, SE33 (ACTBP2), D6S366, D6S474, D6S477, D6S502, D6S965, D6S1017, and D6S1043	D6S132	DQa
7	D7S460, D7S809, D7S820, D7S821, D7S1517, D7S1520, and D7S3048	D7S21, D7S22, D7S467	D7S8 (PM)
8	LPL (LIPOL), D8S306, D8S320, D8S323, D8S344, D8S347, D8S639, D8S1132, D8S1115, and D8S1179		
9	D9S52, D9S302, D9S304, D9S925, D9S1122, and D9S2157		
10	D10S89, D10S1248, D10S1435, D10S2325, and D10S2326	D10S28	
11	APOAI1, TH01 (TC11), UGB, D11S488, D11S554, and D11S4463		HBGG (PM)
12	CD4, PLA2A1, VWA, D12S67, D12S391, D12S1090, and D12ATA63	D12S11	

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Chromosome	STR markers	RFLP-based VNTR markers	Other PCR-based markers
13	D13S308, D13S317, and D13S1492		
14	D14S306, D14S608, and D14S1434	D14S13	
15	CYAR04 (P450), FES/FPS, Penta E, D15S659, and D15S822		
16	D16S537, D16S539, and D16S3253	D16S85	
17	D17S974, D17S976, and D17S1301	D17S79, D17S26	D17S5, YNZ22
18	MBP, D18S51, D18S535, D18S849, D18S853, and D18S1270		
19	D19S253, D19S433		LDLR (PM)
20	D20S85, D20S161, D20S470, D20S482, and D20S1082		
21	Penta D, D21S11, D21S1437, and D21S2055		
22	D22S683 and D22S1045		
X	HPRTB, ARA, STRX1, DXYS156, DXS101, DXS981, DXS6789, DXS6795, DXS6797, DXS6800, DXS6801, DXS6803, DXS6807,DXS6809, DXS7130, DXS7132, DXS7133, DXS7423, DXS7424, DXS8377, DXS8378, DXS9895, DXS9898, DXS9905, DXS9908, DXS10011, GATA31E08, GATA165B12, and GATA172D05		Amelogenin
Y	DYS19, DXYS156, DYS385 a/b, DYS388, DYS389 I/II, DYS390, DYS391, DYS392, DYS393, DYF406S1, DYS437, DYS438, DYS439, DYS444, DYS446, DYS447, DYS448, DYS449, DYS456, DYS458, DYS460, DYS461, DYS462, DYS463, DYS464 a/b/c/d, DYS481, DYS485, DYS495, DYS505, DYS508, DYS520, DYS522, DYS525, DYS531, DYS532, DYS533, DYS534, DYS540, DYS549, DYS556, DYS557, DYS570, DYS576, DYS578, DYS589, DYS594, DYS617, DYS635, DYS643, DYS724 (CD a/b), GATA-H4, and YCAII a/b		Amelogenin and Y-SNPs
mtDNA			HV1 and HV2

Table 1. Chromosome location of mini- and microsatellites [72].

The method is semiquantitative and has a moderate level of sensitivity—1–5%; neither gender nor HLA compatibility of donor and recipient can influence its informativity; it requires a very small number of cells, which makes it applicable in the first postSCT days.

Multiplex platforms use STR/VNTR (variable number tandem repeats) loci with identical amplification protocols and different allelic lengths, using fluorescently labeled primers. Due to its large size, VNTR more often showed discrepancies in the evaluation of chimerism between markers [73]. All loci are amplified together in a single PCR reaction and amplification products are electrophoretically separated on an automated DNA sequencer—fragment analysis. Further analysis is carried out by specialized software. Microsatellites are more widely used, since they are polymorphic, more sensitive, faster to work with, and cheaper. Despite their excellent performance in forensic science, their application in the study of chimerism has its limitations. The most important one results from the very design of the platform—the amount of DNA is indirectly evaluated based on measurement of fluorescence during electrophoresis. The various fluorophores differ in their efficiency to emit light, and the platform has no internal calibration for measurement. As a result, the measurement of one and the same absolute value of DNA will not be equally estimated in all loci. However, this fluorescence-based technology is considered the gold standard in the study of chimerism [58]. Another feature of the STR system is the presence of additional signals—stutter peaks, which are mainly n − 1 and to a lesser degree n + 1 signals [74, 75]. These artifacts are the result of slipped-strand mispairing during amplification. Their size depends on the size and type of the allele, and is in the range 2–13% of their respective base peaks. Stutter is less pronounced with larger repeat units (dinucleotides > tri- > tetra- > penta-). Longer repeat regions generate more stutter, and each successive stutter product is less intense. If stutter peaks match the corresponding donor or recipient peaks, this would affect the estimate of chimerism. This is especially important at low levels of the host residual cells, which would hamper the assessment of minimal residual disease. In heterozygous loci, the two alleles should be equal in amount, but due to stochastic effects during PCR amplification, an imbalance in the two detected alleles is established—this is especially true when the amount of DNA being amplified is limited. Under conditions of extreme imbalance, one allele may "drop-out".

6. Conclusion

Different protocols have been used to condition patients prior to HSCT. They differ from each other in intensity of myeloablation. Myeloablative conditioning is very aggressive and leads to high toxicity and TRM, so its application is limited to younger patients and patients in good medical condition. The risk of TRM decreases with time, although the causes for this are not fully understood. Most likely this is due to advances in technology for HLA typing, better understanding of the role of HLA compatibility, particularly with regard to unrelated donors, and better medical services for patients. Various conditioning protocols are deemed to have varying degrees of ablation of the recipient immune system resulting in different profiles of posttransplantation recovery.

The levels of chimerism after HSCT depend on a number of factors, including the intensity of pretransplant conditioning, the use of T cell-depleted grafts, the number of transplanted stem cells, the sensitivity of the technique used for detection and the interval of study, and as well as the type of the disease.

The main objective in posttransplantation monitoring is early diagnosis of adverse events. Due to the dynamic nature of the development of chimerism, its monitoring should be carried out at short intervals, especially in the first 1–2 months after transplantation, when the risk of disease recurrence or graft failure/rejection is greatest. In the earliest posttransplantation period, the presence of persistent mixed chimerism or the disappearance of donor alleles are associated with both graft rejection and early relapse of the underlying disease. On the other hand, the increasing number of recipient cells in the later periods after the transplantation is associated with upcoming relapse or late allograft rejection. The mechanisms that direct hematopoietic recovery toward one or another course are not yet fully understood. The analysis of chimerism kinetics allows early differentiation between the lack of engraftment and its delay, as well as early detection of patients at high risk of developing GVHD or susceptible to a relapse. In this context, the study of chimerism is undoubtedly an important method for monitoring the outcome of HSCT.

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