Biol Blood Marrow Transplant 23 (2017) 1658–1668

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

ASBMT American Society for Blood and Marrow Transplantation

Clinical Utility of Quantitative PCR for Chimerism and Engraftment Monitoring after Allogeneic Stem Cell Transplantation for Hematologic Malignancies

Müberra Ahci [1], Karin Stempelmann [1], Ulrike Buttkereit [2], Pietro Crivello [1], Mirko Trilling [3], Andreas Heinold [4], Nina Kristin Steckel [2], Michael Koldehoff [2], Peter A. Horn [4], Dietrich W. Beelen [2], Katharina Fleischhauer [1,5,*]

[1] *Institute for Experimental Cellular Therapy, University Hospital Essen, Essen, Germany*
[2] *Department of Bone Marrow Transplantation, West German Cancer Center, University Hospital Essen, Essen, Germany*
[3] *Institute of Virology, University Hospital Essen, Essen, Germany*
[4] *Institute for Transfusion Medicine, University Hospital Essen, Essen, Germany*
[5] *German Cancer Consortium (DKTK), Heidelberg, Germany*

Article history:
Received 6 March 2017
Accepted 30 May 2017

Key Words:
Chimerism
Quantitative PCR
Engraftment
Relapse
Cytomegalovirus
Donor lymphocyte infusion

A B S T R A C T

Although quantitative PCR (qPCR) has been explored for chimerism monitoring after allogeneic stem cell transplantation (SCT), evidence regarding its clinical utility compared with standard short tandem repeat (STR) is still limited. We retrospectively studied commercial qPCR and STR chimerism with respective positivity thresholds of .1% and 1% in 359 peripheral blood (PB) and 95 bone marrow (BM) samples from 30 adult patients after first HLA-matched SCT for myeloid malignancies or acute lymphatic leukemia. Concordance between the 2 methods was 79.5%, with all discordant samples positive in qPCR but negative in STR. Of the latter, sporadic qPCR positivity without clinical correlates was seen mostly in BM samples early post-transplant. In 7 of 21 patients with available follow-up samples in the first months after transplantation, qPCR but not STR revealed low levels (<1%) of sustained host chimerism in PB, reflecting delayed engraftment or persistent mixed chimerism (PMC). These conditions were associated with donor–recipient cytomegalovirus (CMV) serostatus and early CMV reactivation but not with immunosuppressive regimens or clinical outcome. qPCR predicted all 8/8 relapses with samples in the 6 months before onset by sustained positivity in both PB and BM compared with 1/8 relapses predicted by STR mainly in BM. The response kinetics to donor lymphocyte infusions for the treatment of PMC or relapse was shown by qPCR but not STR to be protracted over several months in 3 patients. Our results demonstrate the superior clinical utility of qPCR compared with STR for monitoring subtle changes of host chimerism associated with different clinical conditions, making a case for its use in the clinical follow-up of transplant patients.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (SCT) is a powerful therapeutic tool for a variety of oncohematologic diseases, including high-risk leukemia, with over 16,000 such transplants performed in 2014 in Europe alone [1-4]. The most commonly used stem cell source consists of mobilized peripheral blood stem cells from matched unrelated donors (MUDs), followed by HLA-matched or -mismatched family donors and umbilical cord blood [3]. The preparative regimen can be either myeloablative conditioning or reduced-intensity conditioning, the former leading more frequently to a status of full donor hematopoietic chimerism (HC) compared with the latter [5].

A status of persistent mixed chimerism (PMC) of blood cells from donor and recipient post-transplant is a possible outcome in particular after SCT for nonmalignant disorders such as inborn hemoglobinopathies [6,7]. Although PMC can degenerate into graft failure with the eventual return of full patient chimerism, long-term coexistence of patient and donor blood cells can also be achieved and is frequently associated with the development of a status of immunologic tolerance mediated by regulatory T cells [7,8]. On the other hand, depletion of donor T cells either by ex vivo manipulation of the graft or in vivo by administration of ablative agents such as

Financial disclosure: See Acknowledgments on page 1667.
* Correspondence and reprint requests: Katharina Fleischhauer, MD, Institute for Experimental Cellular Therapy, University Hospital Essen, Hufelandstrasse 55, 45122 Essen, Germany.
E-mail address: katharina.fleischhauer@uk-essen.de (K. Fleischhauer).

http://dx.doi.org/10.1016/j.bbmt.2017.05.031
1083-8791/

antithymocyte globulin (ATG) or alemtuzumab as part of immune prophylaxis can be used for the prevention of rejection and graft versus host disease (GVHD) but has also been associated with delayed immune cell reconstitution [9,10].

Timely and efficient donor stem cell engraftment is an important prerequisite for the ultimate clinical success of allogeneic SCT, both in terms of protective immunity against pathogens to reduce infection-related nonrelapse mortality and in terms of the graft-versus-leukemia effect mediated by the donor's immune system against residual patient-derived malignant cells [9]. Among the common post-transplant infectious complications is reactivation of cytomegalovirus (CMV), a herpes virus latent mainly in blood tissues of the host [11]. Depending on different factors, including T cell depletion and the pre-existing anti-CMV immunity in patient and donor, CMV reactivation occurs in up to 60% of patients after allogeneic SCT and, if not controlled by antiviral therapy, can cause overt CMV disease with severe clinical symptoms and elevated nonrelapse mortality [12-14]. On the other hand, CMV reactivation has also been associated with reduced risk of post-transplant recurrence of acute myeloid leukemia (AML) [15-20], 1 of the most frequent indications for allogeneic SCT [4]. This association, however, is debated and could be modulated by the use of T cell depletion [14,21-23].

Relapse is 1 of the major impediments to the success of allogeneic SCT for hematologic malignancies, in particular acute leukemias, occurring in at least one-third of patients depending on different clinical variables, including diagnosis and disease status at transplant [24]. Detection of impeding relapse at a molecular level is a major challenge that needs to be overcome to allow for efficient pre-emptive treatment. Unfortunately, in particular high-risk AML treated by allogeneic SCT frequently lacks a tumor-specific marker for monitoring of minimal residual disease (MRD) [25]. Because most relapses post-transplant are of patient origin [26], patient-specific genomic polymorphisms can serve as surrogate for tumor-specific MRD markers in early relapse detection. Such polymorphisms are targeted in the analysis of HC after transplantation, which thus serves the double purpose of monitoring engraftment kinetics and relapse. In contrast, HC has a completely different significance in HSCT for nonmalignant disease such as severe aplastic anemia, where relapse of malignant cells is not an issue but graft rejection is a major concern. In this setting, sensitive engraftment monitoring can provide useful information for early detection and possibly prevention of this complication.

The current gold standard for HC monitoring in clinical routine is short tandem repeat (STR) analysis, based on PCR amplification of different STR loci that vary by 1 to several base pairs in length between different individuals [27-29]. PCR products of different sizes are resolved by capillary gel electrophoresis, and the relative amount of patient and donor cells in the original sample is determined by semiquantitative analysis of the area under the peak of patient- or donor-specific amplicons. This method was originally developed for forensic purposes and has several advantages, including a high level of standardization, robustness, and time and cost efficiency. A drawback of the method, however, is its intrinsically limited sensitivity of 1% to 5% [30-32], because of the need to keep the amount of target DNA to a minimum of few nanograms to avoid PCR competition and plateau biases. This problem has more recently been overcome by real-time quantitative PCR (qPCR), a directly quantitative method evaluating the cycle threshold, which is inversely proportional to the original amount of target DNA [33]. For HC analysis, the cycle threshold of the gene of interest is compared in reference with an internal housekeeping gene and with the patient DNA pretransplant (delta-delta cycle threshold method). The amount of input DNA is flexible and directly proportional to the sensitivity of qPCR, which at 100 ng is more than 2-log higher than that of STR.

The first methods for qPCR-based HC determination on single nucleotide polymorphisms or insertion deletion polymorphisms have been described over a decade ago [34-36], and several commercial kits are currently available for this purpose. The feasibility and enhanced sensitivity of this system compared with STR has been documented in different studies [32,37-40]. Most reports addressing the clinical utility of qPCR HC have focused on the endpoint disease relapse, which was shown to be detected significantly earlier by qPCR than by STR [41-43]. Consensus is still missing on the best cut-off value for positivity in qPCR as well as the preferable use of bone marrow (BM) or peripheral blood (PB), the latter with obvious logistical advantages for sample acquisition at sufficiently high abundance but with potentially lower informative value compared with BM, the natural environment for relapse onset. Moreover, only a single report has to our knowledge so far addressed the question of engraftment monitoring by qPCR, in the particular setting of umbilical cord blood SCT [44]. Possibly because of the still limited available evidence for its clinical utility, most clinical laboratories still do not use qPCR for routine chimerism testing but prefer STR as the standard method.

In the present study we aimed to comparatively investigate the clinical utility of STR and qPCR for engraftment and relapse monitoring after MUD-SCT, the most frequent SCT setting, in a detailed retrospective analysis of 30 clinically well-characterized and informative patients. In this context we were also able to address new questions including the imprinting of CMV reactivation on engraftment as well as the efficiency and kinetics of the response to donor lymphocyte infusions (DLIs) for the treatment of PMC and relapse. Our data provide new and compelling evidence for the added informative value of qPCR over STR as the standard method for chimerism monitoring after allogeneic SCT.

METHODS

Patients and Transplants

Thirty adult patients who received a first allogeneic SCT mainly from unrelated donors for AML, acute lymphatic leukemia, or other myeloid malignancies between 2006 and 2013 at the University Hospital Essen, Germany were included in the analysis. Enrollment criteria included diagnosis, donor type, and the availability of several follow-up samples and their STR chimerism results for retrospective chimerism analysis by qPCR. Patient and donor characteristics, including diagnosis and disease status at transplantation; donor–recipient sex; CMV serostatus; HLA matching status; and conditioning regimen and date of transplant are shown in Table 1. All but 2 patients received myeloablative conditioning, followed by infusion of unmanipulated donor PB stem cells and GVHD prophylaxis based on short-course methotrexate and cyclosporine A for at least 210 days. Immune prophylaxis included ATG (Fresenius; Neovii Biotech, Gräfeling, Germany) at a total dose of 60 mg/kg pre-SCT in 19 patients (Table 1). Transplants were performed after written informed consent, under clinical protocols approved by the Ethical Review Board of the University Hospital Essen, in accordance with the Declaration of Helsinki.

Clinical Outcome Endpoints

The 2 main clinical outcome endpoints of this study were engraftment and disease relapse. Follow-up was recorded until January 29, 2015, with a median time of follow-up of 1504 days (range, 317 to 2981).

Time to engraftment was defined as days post-SCT needed for achievement of at least 500 WBCs per μL. Patients were considered informative for engraftment when follow-up samples were present from different time points in the first 210 days post-SCT and did not present with disease relapse during that time. Engraftment kinetics were classified as *normal* (ie, sustained <.1%