

Real-time PCR for Monitoring Minimal Residual Disease and Chimerism in Patients after Allogeneic Transplantation

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Abstract

Real-time PCR is a new fluorometric method for cycle-to-cycle quantification of PCR product growth rates. The real-time PCR method is fast and associated with a high reproducibility rate. It is used more often for monitoring MRD and chimerism in patients after allogeneic stem cell transplantation (SCT).

There are real-time PCR methods for patients with CML, AML and ALL patients with inv(16), t(8;21), t(15;17); t(1;19) and other chromosomal aberrations. For patients with AML monitoring MRD is useful to identify patients who were at high risk for relapse after receiving chemotherapy. In patients with CML monitoring MRD might be helpful to assess success of after allogeneic SCT, or response to therapies with interferon alfa or STI 571. We found, that it is possible to estimate the relapse stage in CML after SCT by the amount of bcr-abl fusion transcript detected using a real-time PCR method. The median measured bcr-abl amount differ significantly ($P < 0.001$) between the various stages, which has relevant clinical implications because it enables early therapeutic decisions in relapsing patients after transplant as e.g. the application of DLI to induce graft-versus-leukemia effects.

Using real-time PCR it is possible to detect differences at alleles between recipient and donor at a single nucleotide basis (SNP) for chimerism analysis. The real-time PCR method enables to achieve a high a sensitivity of up to 1×10^{-4} , which is much more sensitive than all other chimerism methods including VNTR-PCR, STR-PCR. Furthermore, chimerism in male recipients with a female donor can be monitored also by detecting y-chromosome specific sequences by real-time PCR after transplant, which might be the most sensitive method to detect host type gene sequences.

All in all, new real-time PCR methods offer a fast, reliable and very sensitive method to evaluate MRD and chimerism in patients after allogeneic SCT and therefore, to help to identify patients who are at high risk for leukemic relapse.

The development of a fluorometric method for cycle-to-cycle quantification of PCR product growth rate as the real-time PCR promises to provide an accurate molecular method for a fast and reliable quantification of nucleic acid sequences. In contrast to current end-point quantification methods such as the competitive PCR assay, the real-time method may be associated with a lower variance in the result combined with a high reproducibility rate. Therefore the real-time PCR methods seems to be suitable for monitoring minimal residual disease and chimerism in patients after allogeneic transplantation.

Up to date, several real-time PCR methods exist al-

ready. Besides the use of hybridization probes for real-time PCR to reflect fluorescent signals, real-time PCR could be performed by use of SYBR green, too. The most common used hybridization probes are TaqmanTM probes, hybridization probes developed for the Light-Cycler[®] device, and molecular beacon[®] probes, which did not differ much in sensitivity and sensibility.

Recently, many real-time PCR assays have been established for the detection of minimal residual disease in patients with acute and chronic leukemia with the most common chromosomal aberrations. So, there are real-time PCR methods for patients with CML detecting the

unique bcr-abl rearrangement gene and real-time PCR methods for patients with AML and ALL patients with inversion 16, translocation t(8;21), t(15;17); t(1;19) and other chromosomal aberrations [1-7]. For patients with AML monitoring MRD by real-time PCR is useful to identify patients who were at high risk for relapse after receiving chemotherapy. In patients with CML monitoring MRD might be helpful to assess success of after allogeneic transplant, or response of therapies with interferon alfa or STI 571 [1,2]. Many studies about the detection of bcr-abl in patients with CML show the feasibility of monitoring MRD using real-time PCR methods. However, we found, that it is possible to estimate the relapse stage in chronic myeloid leukemia after allogeneic stem cell transplantation by the amount of bcr-abl fusion transcript detected using a real-time PCR method. In 402 samples from 172 patients, bcr-abl expression was determined and normalized using the GAPDH housekeeping gene product as an endogenous reference. The median normalized bcr-abl amount differ significantly ($P < 0.001$) between the various stages and was 0,06% (range 0.001-1,5%, 3,2% (range 1.4 5,6%) and 21,5% (range 6,6-827%) in 17 patients with a molecular relapse, 8 patients with a cytogenetic relapse and in 10 patients with a hematologic relapse, respectively [1]. The ability to distinguish between molecular and cytogenetic relapse has relevant clinical implications because it enables early therapeutic decisions in relapsing patients after transplant as e.g. the application of donor leukocyte infusion or withdrawal of immunosuppression to induce graft versus leukemia effects.

Real-time PCR can be used also for the detection of chimerism after transplant. The detection of host-type hematopoiesis after transplant is commonly accepted to be associated with an increased risk for leukemic relapse. Moreover, monitoring the kinetics of mixed chimerism of patients with quantitative chimerism analysis may offer the best way to identify patients at high risk for relapse after transplant.

Using real-time PCR it is possible to detect differences at alleles between recipient and donor at a single nucleotide basis (SNP). Single nucleotide polymorphism exist at various gene locations. The real-time PCR method enables to achieve a high a sensitivity of up to 1×10^{-4} , which is much more sensitive than all other chimerism methods including VNTR-PCR, STR-PCR which have a sensitivity of 1×10^2 up to 1×10^3 only. But chimerism

analysis using real-time PCR is not only more sensitive, it enables in contrast to current end-point quantification methods using gene scanner also a fast and reliable quantification of host type nucleic acid sequences. So it is possible to monitor patients at high risk for relapse much better and relapse can be detected also earlier, which has also relevant clinical implications.

Besides real-time PCR for SNP evaluations for chimerism analysis, male recipients with a female donor can be monitored for the residual host type hematopoiesis also by detecting y-chromosome specific sequences by real-time PCR after transplant, which might be the most sensitive method to detect host type gene sequences.

All in all, new real-time PCR methods offer a fast, reliable and very sensitive method to evaluate MRD and chimerism in patients after allogeneic transplant and therefore, to help to identify patients who are at high risk for leukemic relapse.

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