Chip-based digital PCR: an accurate and sensitive method for routine chimerism monitoring after hematopoietic stem cell transplantation.

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BACKGROUND

After hematopoietic stem cell transplantation (HSCT) recipient and donor populations of leukocytes cohabit in the peripheral blood of the patient, resulting in "chimerism". Monitoring chimerism is crucial to follow the outcome of the disease and to make informed clinical decision concerning further therapeutic interventions. Current chimerism monitoring is performed using Short-Tandem-Repeats analysis (STR) and quantitative real-time PCR (RT-PCR). However, both methods suffer from limited sensitivity and reproducibility. Digital PCR (dPCR) represents a new alternative for accurate and reproducible chimerism monitoring.

METHODS

Chip-based Quantstudio 3D chip-based dPCR (Applied Biosystems, Reinach, Switzerland) allows for SNP detection in approximately 20'000 separate RT-PCRs per sample, with statistical presence of only 0, 1, or 2 DNA molecules per each 865 pL reaction well. Fluorescence of each well is separately measured and delivers absolute counts of the two different alleles. Original samples were analyzed for their SNP genotypes by PCR using Sequence Specific Priming (PCR-SSP).

RESULTS

Using artificial DNA mixes, we estimated our limit of detection to range at approximately 0.5 % for the minor DNA. Results within the same samples were highly homogeneous using six different SNP-Taqman assays for evaluation. Typing samples of Instand's external proficiency testing (EPT) for post-HSCT chimerism, was performed on one pre-HSCT patient and donor sample each and on five post-HSCT samples, using only two different SNP-Taqman assays. Instand honored our result quality with 20/20 points. On 15 DNA sample triplets, consisting of one pre-HSCT, one donor and one post-HSCT specimen each, dPCR results were consistent with previous STR results in 11 out 15 triplets. Discrepancies were only observed for 4 triplets, where chimerism was estimated to range below 5 % by STR analysis, a method with known limited sensitivity (Stahl et al., Exp Hematol, 2015 Jun).

CONCLUSION

According to the high performance of our method in the Instand EPT and based on the results of 15 patient/donor/post-HSCT sample triplets, chip-based dPCR appears as an accurate and routinely applicable technique for exact chimerism determination with excellent sensitivity.