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Minimal Residual Disease Detection by Droplet Digital PCR in Multiple Myeloma, Mantle Cell Lymphoma, and Follicular Lymphoma

A Comparison with Real-Time PCR

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Real-time quantitative PCR (qPCR) is a well-established tool for minimal residual disease (MRD) detection in mature lymphoid malignancies. Despite remarkable sensitivity and specificity, qPCR has some limitations, particularly in the need for a reference standard curve, based on target serial dilutions. In this study, we established droplet digital PCR (ddPCR) for MRD monitoring in multiple myeloma, mantle cell lymphoma, and follicular lymphoma and compared it head-to-head with qPCR. We observed that ddPCR has sensitivity, accuracy, and reproducibility comparable with qPCR. We then compared the two approaches in 69 patients with a documented molecular marker at diagnosis (18 multiple myelomas, 21 mantle cell lymphomas assessed with the immunoglobulin gene rearrangement, and 30 follicular lymphomas with the use of the BCL2/immunoglobulin gene major breakpoint region rearrangement). ddPCR was successful in 100% of cases, whereas qPCR failed to provide a reliable standard curve in three patients. Overall, 222 of 225 samples were evaluable by both methods. The comparison highlighted a good concordance ($r = 0.94$, $P < 0.0001$) with 189 of 222 samples (85.1%; 95% CI, 80.4%—89.8%) being fully concordant. We found that ddPCR is a reliable tool for MRD detection with greater applicability and reduced labor intensiveness than qPCR. It will be necessary to authorize ddPCR as an outcome predictor tool in controlled clinical settings and multilaboratory standardization programs. *(J Mol Diagn 2015, 17: 652—660; http://dx.doi.org/10.1016/j.jmoldx.2015.05.007)*

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Detection of minimal residual disease (MRD) allowed acquisition of valuable prognostic information in several mature lymphoid malignancies with a considerable impact on clinical research.[1,2] Currently, it is often included as a secondary end point in clinical trials for multiple myeloma (MM), mantle cell lymphoma (MCL), and follicular lymphoma (FL).[3–5] More recently, several cooperative groups have designed MRD-based risk-adapted studies in a number of therapeutic settings.[6]

Different methods can be used for MRD quantification, including flow cytometry (FC),[7–9] real-time quantitative PCR (qPCR),[10–13] and the more recent next-generation sequencing (NGS).[14,15] So far, qPCR remains the most validated and standardized method in MCL and FL.[4,5] In MM, for which FC also has a major role,[16] the International Myeloma Working Group has included molecular complete response (tumor marker negativity by PCR at sensitivity 10^{-5}), as a meaningful criterion for response evaluation.[17] In MM and MCL, qPCR uses immunoglobulin gene (*IGH*) rearrangement as a clonal marker, whereas in FL the most reliable marker is the t(14;18) translocation, especially when the major breakpoint region (*BCL2/IGH MBR*) is involved.[18]

qPCR represents the most widely used method for MRD analysis. However, it has a major limitation from being a relative quantification approach. This results in the need of a reference standard curve usually built by dilutions of the tumor-specific target obtained from diagnostic DNA, plasmids, or cell lines that contain the rearrangement of interest. Moreover, qPCR is unable to provide reliable target quantification for a substantial proportion of samples that have a tumor burden between the sensitivity and the quantitative range of the method. Samples that fall in this window of inadequate quantification, which might range up to two logs and are sometimes difficult to categorize for clinical purposes, are usually defined as positive nonquantifiable (PNQ).[19]

Droplet digital PCR (ddPCR) is based on sample compartmentalization in single oil droplets that represent independent PCR reactions and on end point amplification and Poisson statistics.[20–24] ddPCR has several theoretical advantages compared with qPCR,[25–29] most notably allowing for absolute quantification of target DNA molecules and avoiding the need for a reference standard curve; thus, it is potentially valuable in the MRD setting.

On the basis of these considerations, we sought to verify the utility of ddPCR as a MRD monitoring tool and to compare it head-to-head with qPCR in 69 patients, including 18 with MM, 21 with MCL, and 30 with FL for a total of 225 samples. Our aim was to verify whether ddPCR could overcome some limitations of qPCR without losing its critical advantages, especially in terms of sensitivity and reproducibility.

Materials and Methods

Sample Characteristics and DNA Extraction

Preliminary evaluation of ddPCR performance was conducted with plasmid and purified neoplastic cell dilutions for the *IGH* rearrangement and the DOHH-2 cell line for the *BCL2/IGH MBR*, as previously reported.[10,30,31] For method comparison, genomic DNA (gDNA) derived from bone marrow (BM) and peripheral blood (PB) samples from 69 patients (18 with MM, 21 with MCL, and 30 with FL) was used. Samples were selected for having a molecular marker on the basis of the *IGH* (MM and MCL) or *BCL2/IGH MBR* (FL) rearrangements and were collected in the context of prospective clinical trials approved by the local institutional review board (MCL: EUdract2009-012807-25; MM: Eudract2004-000531-28 and Eudract2008-008599-15; FL: Eudract2009-012337-29). All patients provided written informed consent, which included PCR-based MRD determination, according to the Helsinki Declaration. Overall, 225 samples (180 BM and 45 PB) were analyzed: 95 MM, 70 MCL, and 60 FL. A total of 70 were diagnostic samples [for one patient two diagnostic samples (BM, PB) were available], and 155 were taken during patient follow-up on the basis of availability of DNA (Supplemental Table S1). MCL and FL sample mononuclear cells were separated by density gradient (Histopaque-1077; Sigma-Aldrich, St. Louis, MO), whereas MM samples were treated with erythrocyte lysis buffer. gDNA was extracted, depending on the amount of cells, by DNAzol (Life Technologies-Invitrogen, Carlsbad, CA) or NucleoSpin Tissue (Macherey-Nagel, Bethlehem, PA), according to the manufacturer's recommendations. gDNA quality and concentration were estimated by Nanodrop 2000C (Fisher Thermo Scientific, Waltham, MA) before experimental use. To avoid possible biases related to sampling, qPCR and ddPCR quantification were performed on the same diluted gDNA samples. Detailed information is included in Supplemental Table S2, as suggested by the guidelines for the Minimum Information for the Publication of Digital PCR Experiments (dMIQE).[32]

Tumor-Specific Molecular Marker Assessment

In MM and MCL, patient-specific *IGH* rearrangements were amplified and direct sequenced from diagnostic gDNA.[10,31] Sequences were analyzed with the IMGT/V-QUEST tool (*http://imgt.org/IMGT_vquest/share/textes*, last accessed March 26, 2015),[33,34] and patient-specific allele-specific oligonucleotide primers and consensus probes were designed as previously described.[10] FL patients were screened at diagnosis for the *BCL2/IGH MBR* translocation, as already described.[18]

qPCR

IGH-based and *BCL2/IGH* MBR-based MRD detection by qPCR was performed with an AbiPrism7900HT (Life Technologies-Applied Biosystems, Carlsbad, CA), as previously described.[18,19] For each patient, sample estimation was based on serial 10-fold dilution standard curves, prepared according to Euro-MRD guidelines, as previously