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First comparison between Multicolor Flow Cytometry and droplet digital PCR for tumor burden quantification at baseline in mantle cell lymphoma

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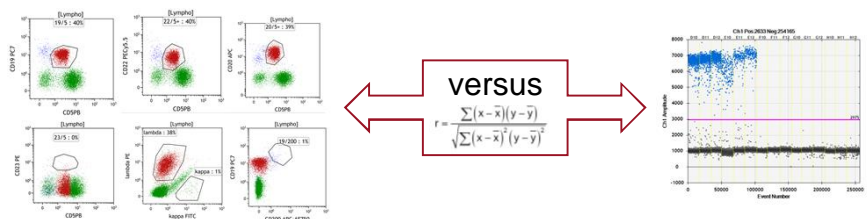
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BACKGROUND AND AIMS

Quantification of tumor load at diagnosis has been shown to provide an additional prognostic tool, in mature lymphoproliferative disorders [1]. Multiparameter Flow Cytometry (MFC) is the most commonly used method to assess the degree of tumor infiltration at baseline. However, inter-laboratory standardization still needs to be fulfilled before MFC can be implemented in multicenter trials. **Since droplet digital PCR (ddPCR) represents a feasible alternative, effortless to standardize and potentially able to overcome some MFC drawbacks, we compared the reliability of ddPCR versus MFC for tumor quantification at baseline, in a phase III, multicenter clinical trial for mantle cell lymphoma (MCL) patients.**

METHODS

ddPCR was performed on baseline samples of MCL patients enrolled in the Italian %MCL0208+trial (EUdract:2009-012807-25) sponsored by FIL (Fondazione-Italiana-Linfomi). Quantification of IGH-VDJ by ddPCR was performed with the QX100 ddPCR system (Bio-Rad) as described [2]. The same allele-specific primers and consensus probes set up for qPCR were used in ddPCR. 500 ng of gDNA were loaded in triplicate, a negative control (gDNA pooled from 10 healthy donors) and NTC were included. The final tumor load was calculated as the merge of replicates. MFC was performed by a 6-color panel for BM (K/L/CD19/CD23/CD5) and PB (also CD22/CD20/CD43/CD200) on FACSCantoll (Becton Dickinson) (Fig1). qPCR was based on serial 10-fold dilution standard curves, starting from 500ng gDNA, using a AbiPrism7900HT (Life Technologies), according to Euro-MRD guidelines [3]. Methods comparison was assessed using bivariate Pearson correlation and results were considered discordant when difference in clonal cells quantification was >=1 log.



RESULTS

MFC versus ddPCR, was performed on 131 baseline samples, 64 Bone Marrow (BM) and 67 Peripheral Blood (PB), from 64 MCL patients. Overall, the correlation between the two techniques was moderate (r=0,6) and slightly higher for PB samples (r=0,7) (Figure1). Stratifying the samples based on MFC tumor infiltration (27 low, <1%, 45 mid, 1-10% and 59 high, 10-100%), Pearson correlation show a better correlation for samples with MFC>10% (PB r=0,5 and BM r=0,6) compared to samples with low MFC<10% (PB r=0,3, BM r=0,01). Superimposable results with ddPCR were observed in terms of median of absolute target copies, low: 253 (range 2-27993), mid:1636 (2-66000) and high: 16133 (14-72550), however, the leukemic nature of MCL was better reflected by ddPCR (PB:7068 copies, range 2-56387, BM:3100 copies; range 4-66000) compared to MFC (PB:6%, range 0,01-92,6; BM:8,7%, range 0,03%-92,1%).

33/131(25%) samples were discordant, not clustering in specific patients or tissues. Notably, 24% of discordances (8/33: 4 low, 3 mid, 1 high tumor infiltration), showing ddPCR>MFC, were confirmed by qPCR, suggesting a MFC underestimation, probably due to sample shipment modalities in a multicenter trial. However, in other 24% highly infiltrated samples (8/33: 1 mid, 7 high) qPCR endorsed the MFC results, discrepancy that might be related to the use in qPCR, and not in ddPCR, of 10 times diluted DNA. It must be speculate that saturation issue of ddPCR reaction could have been affected the reliability of the quantification, in highly infiltrated samples. Unfortunately, in half of the discordant cases (17/33, 52%, 7 low, 9 mid, 1 high) the qPCR performance was suboptimal, consequently we cannot rely on qPCR data for further inference (Table1).

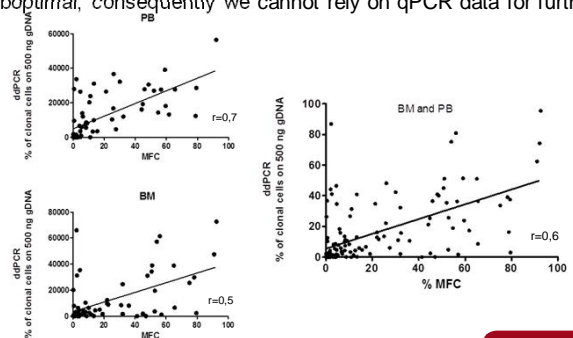


Figure 1

DISCORDANCES

	ddPCR higher than MFC (ddPCR concordant with qPCR- qPCR not available)	ddPCR lower than MFC (ddPCR concordant with qPCR- qPCR not available)
TOT 33		
MFC<=1%	7 (4-3)	4 (0-4)
1%<MFC<=10%	4 (3-1)	9 (1-7) [#]
MFC>10%	1 (1-0)	9 (1-1) [*]

Table 1

7 (*) and 1 (#) samples in which qPCR endorses MFC results

CONCLUSIONS

- “ This study represents the first comparison between MFC and ddPCR, for baseline tumor load quantification in a multicenter MCL trial.
- “ A moderate correlation (r=0,6) between methods has been observed.
- “ Accurate guidelines for ddPCR setting and data analysis are required before this tool can be implemented in the clinical practice.
- “ However, given the high precision, the technical simplicity and the potentiality to be easily standardized, ddPCR might be proposed for tumor load quantification in those cases lacking reliable MFC and/or qPCR data.

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