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

Original
First comparison between Multicolor Flow Cytometry and droplet digital PCR for tumor burden quantification at baseline in mantle cell lymphoma / Drandi, Daniela; Jimenez, Cristina; Monitillo, Luigia; Barbero, Daniela; Ruggeri, Marina; Mantoan, B; Genuardi, Elisa; Gilestro, Milena; Zaccaria, Gian Maria; Ghione, Paola; Vasta, M; Loschirico, M; Omedè, Paola; Cavallo, Federica; Cortelazzo, Sergio; Boccadoro, Mario; García-Sanz, R; Ladetto, Marco; Ferrero Simone. - (2016), pp. 82-83. ((Intervento presentato al convegno 5th ESLHO symposium tenutosi a Prague.

Availability:
This version is available at: 11583/2662554 since: 2017-06-26T15:58:53Z

Publisher:
European Scientific Foundation of Laboratory Hemato Oncology

Published
DOI:

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BACKGROUND AND AIMS

Quantification of tumor load at diagnosis has been shown to provide an additional prognostic tool, in mature lymphopoietic 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, efforts 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 MCL20[08] trial. MFC was performed by FIL (Fondazione-Italiana-Linfomi). QPCR was performed with the QX100 ddPCR system (Bio-Rad) as described [2]. To 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 (KL/CD19/CD23/CD5) and PB (also CD22/CD20/CD43/CD200) on FACSCantor (Becton Dickinson) (Fiqu1). ddPCR was based on serial 10-fold dilution standard curves, starting from 500ng gDNA, using a ABI Prism 7900HT (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) (Figure 1). 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: 225 (range 2-27993), mid: 1636 (2-66000) and high: 1613 (14-72550), however, the leukemic nature of MCL was better reflected by ddPCR (Figure 2): 7258 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%).

DISCORDANCES

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) (Figure 1). 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: 225 (range 2-27993), mid: 1636 (2-66000) and high: 1613 (14-72550), however, the leukemic nature of MCL was better reflected by ddPCR (Figure 2): 7258 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%).

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.