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Impact of Hemodilution on Flow Cytometry Based Measurable Residual Disease Assessment in Acute Myeloid Leukemia

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Introduction

- Measurable residual disease (MRD) assessed from the bone marrow (BM) of acute myeloid leukemia (AML) patients after induction chemotherapy is an established prognostic factor[1]
- Hemodilution, the mixing of peripheral blood (PB) within BM during aspiration, can yield false-negative MRD results[2]
- Various parameters that represent cellular BM/PB composition and formulas have been proposed to quantify or compensate for hemodilution in AML and other hematologic diseases, however, the exact impact of hemodilution remains unknown[3]

Aim

- To prospectively assess the impact of hemodilution over different pulls of BM as representative for conventional aspiration
- Furthermore, to evaluate the sensitivity and specificity of previously reported hemodilution formulas to estimate and/or correct hemodilution

Methods

- Inclusion criteria: AML patients aged 18 years or older undergoing high-dose chemotherapy following HOVON-SAKK guidelines
- A BM aspirates were obtained from three consecutive 2 mL pulls (pull 1 (first 2ml BM), pull 2 (second 2ml BM), pull 3 (third 2 ml BM), along with PB samples obtained on the same day
- A total of 30 patients were included, rendering 120 samples
- Using MFC, the leukemia-associated phenotype (LAIP) was identified at diagnosis in BM and analyzed with either 6- or 8-color antibody panels[4]
- MRD is given as a percentage of the total white blood cell (WBC) count
- A Friedman test compared percentages of blasts and MRD among pulls and PB. Differences in outcomes across specimens were assessed using Friedman's ANOVA test. The ability of individual features and/or populations to discriminate between BM and PB samples was evaluated in a binary classification task using the area under the receiver operating characteristic curve (ROC-AUC)

References

1. Short *et al*, JAMA Oncology 2020
2. Blachly *et al*, Haematologica 2022
3. Tettero *et al*, HemaSphere 2021
4. Cloos *et al*, J Vis Exp 2018
5. Delgado *et al*, J Immunol Methods 2017
6. Flores-Montero *et al*, Leukemia 2017

Results

A statistical significant decrease in MRD% between pull 1-3 and PB as reference

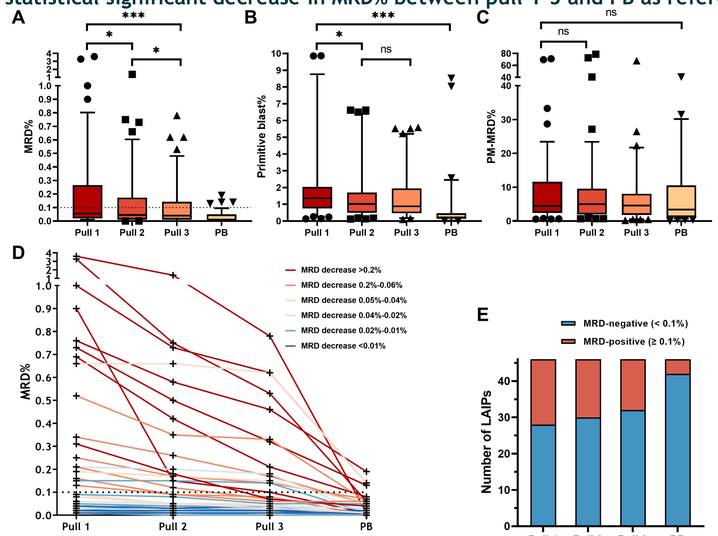


Figure 1. (**A**) Differences in MRD% between the different pulls and peripheral blood (PB). Differences between pull 1 and pull 2 (p=0.025), pull 1 and pull 3 (p<0.001), pull 2 and pull 3 (p=0.025) and between all pulls and PB (p<0.001) were statistically different. (**B**) Differences in primitive blasts (CD45+ cells with a primitive marker being CD34+, CD117+ or CD133+) between the three pulls and PB. (**C**) Difference in primitive marker MRD (PM-MRD) depicted as the percentage of LAIP cells with the primitive cells as denominator showed no statistical differences between the pulls. (**D**) Consecutive MRD results of the individual successive pulls and PB. Colors indicate level of absolute decrease between pull 1 and pull 3. (**E**) In the 40 paired samples, a total of 46 different LAIPs were identified. Based on the 0.1% cut-off, 39.1% were positive in the first pull, compared to 34.8% in pull 2 and 30.4% in pull 3. In the PB samples, only 8.7% of the LAIPs were above the 0.1% cut-off.

Discrimination of factors between BM and PB based on the ROC

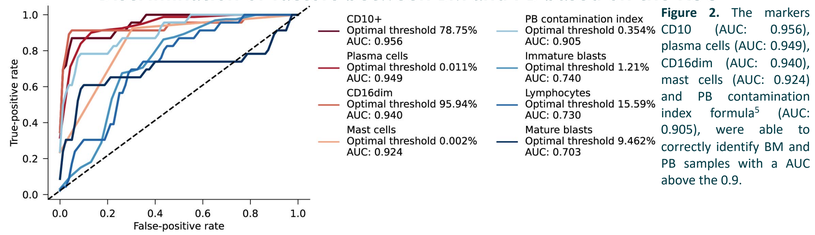


Figure 2. The markers CD10 (AUC: 0.956), plasma cells (AUC: 0.949), CD16dim (AUC: 0.940), mast cells (AUC: 0.924) and PB contamination index formula[5] (AUC: 0.905), were able to correctly identify BM and PB samples with a AUC above the 0.9.

Mast cells significantly decrease with subsequent pulls

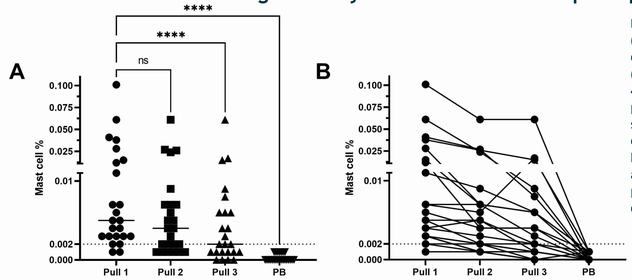


Figure 3. (**A**) Mast cell populations (CD117high) significantly decreased between, pull 1/pull 3 (p<0.001) and pull 1/PB (p<0.001). 4 samples from pull 1, 10 from pull 2, 13 from pull 3, and all PB samples were below the 0.002% cut-off and designated as hemodiluted.[6] (**B**) All samples with a mast cell population <0.002% in pull 1, were also marked as diluted in the subsequent pulls.

Visual example of MRD discrepancy and mast cell decline due to hemodilution

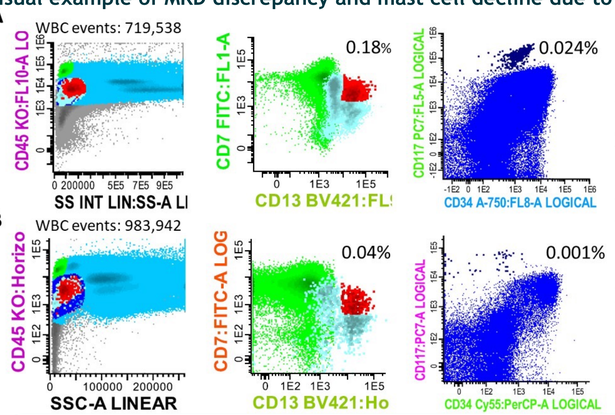


Figure 4. (**A**) The sample assessed at the center where the BM aspiration was performed exhibited an MRD-positive outcome (>0.1%), with a LAIP of CD45+CD13+CD7+ comprising 0.18% of the WBC (shown in red). The mast cell concentration (depicted in dark blue) was determined to be 0.024%. (**B**) At the central laboratory, the exact same LAIP was measured at 0.05% (MRD-negative). The mast cell population in this samples had decreased to 0.001%, indicative of strong hemodilution as the likely cause for the disparity.

Conclusions

- Hemodilution is occurring even after minimal BM aspiration and warrants consideration in MRD assessment
- The mast cell population can be used as a marker for hemodilution
- We advice to send in the first pull for MRD measurement and if impossible, include the tube number in the final MRD report
- In cases of uncertainty, advice to repeat the BM aspiration

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