Received: 13 October 2022 | Accepted: 16 October 2022

DOI: 10.1111/bjh.18533

BJHaem
BRITISH JOURNAL OF HAEMATOLOGY

COMMENTARY

United we stand, divided we fall. Multicentre standardization of measurable residual disease assessment in acute leukaemia is the way forward

Gaurav Chatterjee | **Nikhil Patkar**

Haematopathology Laboratory, ACTREC, Tata Memorial Centre, Navi Mumbai, India

Correspondence
Nikhil Patkar, Haematopathology Laboratory, CCE Building, Advanced Centre of Treatment Research and Education in Cancer, Tata Memorial Centre, Kharghar, Maharashtra 410210, India.
Email: nvpatkar@gmail.com

Assessment of measurable residual disease (MRD) using multiparameter flow cytometry in precursor B-cell acute lymphoblastic leukaemia (ALL) is routine. However, studies on the harmonization of laboratory techniques as well as on the interpretation of results are limited. Here, Ikoma-Colturato and colleagues from Brazil demonstrate multicentric standardization of B-ALL MRD using EuroFlow protocols. Commentary on: Ikoma-Colturato et al., Multicentric standardization of minimal/measurable residual disease in B-cell precursor acute lymphoblastic leukaemia using next-generation flow cytometry in a low/middle-level income country. Br J Haematol 2023;200:381-384.

KEYWORDS
acute lymphoblastic leukaemia, measurable residual disease, multicentre studies, multiparameter flow cytometry, standardization

In their paper, Ikoma-Colturato and colleagues[1] share their experiences in multicentric standardization of a sensitive multiparametric flow cytometry (MFC)-based MRD assay in patients of B-cell precursor ALL (B-ALL) across laboratories in Brazil. MFC-based B-ALL MRD (MFC-BMRD) assays have become popular due to their broad applicability and quick results as compared to molecular techniques. These assays have been integrated into many contemporary treatment protocols. However, the major challenges in the widespread use of MFC-BMRD include variability in sample processing, antibody panels, data analysis and interpretation.[2] Rigorous multicentric standardization is essential in this context, especially in low- and low/middle-income countries (LMIC). Previous successful efforts of large multicentric standardization of MFC-BMRD assays have been published,[3–5] but most of these were based on 4–6-colour MFC. The analysis and interpretation of such assays becomes even more challenging when a significant proportion of regenerative normal B-cell precursors (BCP) is present.

EuroFlow and a few other centres have recently standardized highly sensitive MFC-BMRD assays reaching a sensitivity of 10^{-5} or better.[6,7] A key to achieving such high sensitivity is using well-validated processing protocols to ensure acquiring a large number of cells (>4 million, preferably higher). Moreover, a standardized panel that incorporates optimal antibody–fluorochrome combinations and a set analysis strategy are required to obtain reliable *n*-dimensional separation of residual leukaemic B-blasts from regenerative BCPs (Figure 1). An additional by-product of acquiring a large number of cells is that the normal bone marrow components are also acquired in large numbers. This gives rise to consistent artefacts such as CD19-positive plasmacytoid dendritic cell precursors, CD10-positive transitional B cells, CD19-positive natural killer (NK) cells and others.[8] Therefore, standardization across various centres in processing, acquisition and analysis strategies becomes even more important for the widespread use of MFC-BMRD with high sensitivity. The EuroFlow group has published their protocols, panels and analysis strategies for relatively easy implementation in other centres.[6,9] In their paoer, Ikoma-Colturato et al. have given a blueprint of establishing a well-standardized EuroFlow-recommended highly sensitive BMRD protocol in an LMIC setting.

The authors observed frequent differences in MRD results across various laboratories of Brazil in their earlier effort.[10]

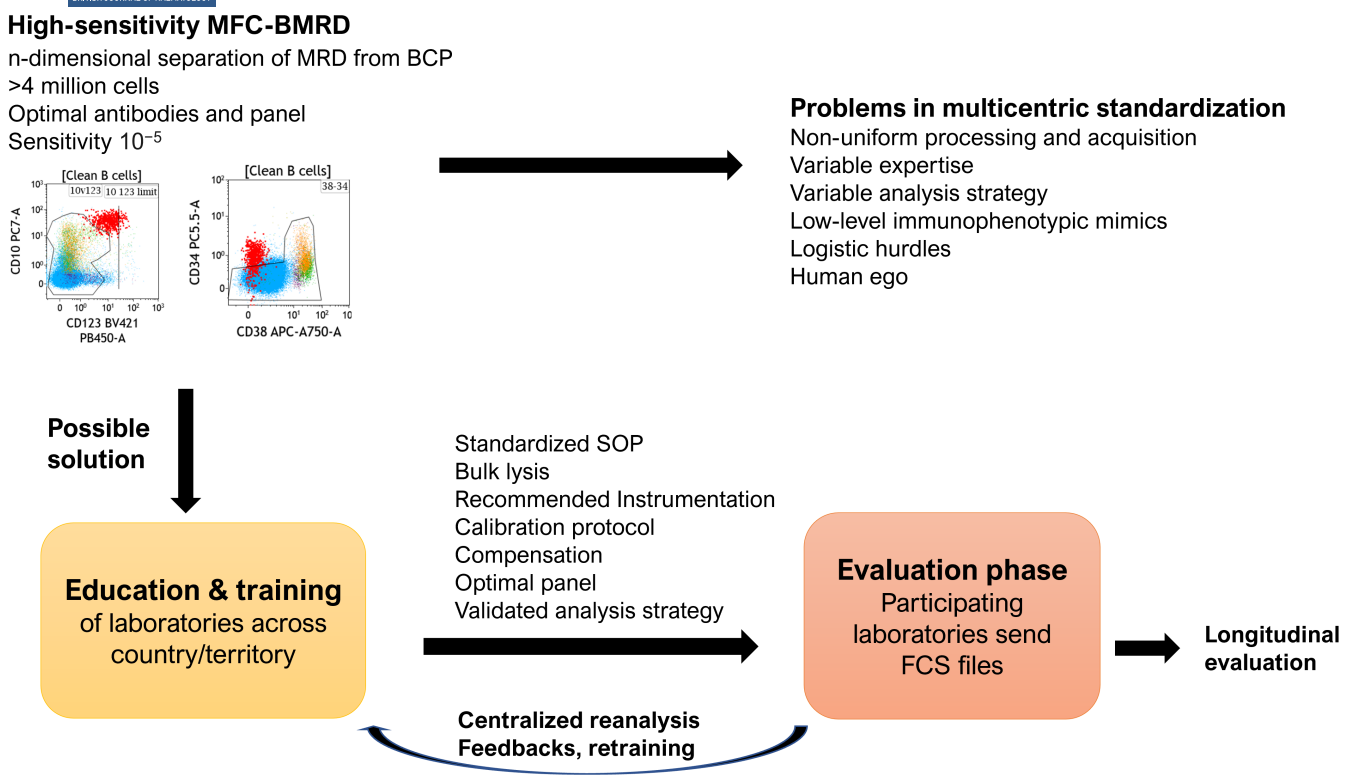

FIGURE 1 Problems and possible solutions for establishing multicentric standardization of a highly sensitive MFC-BMRD assay. [Colour figure can be viewed at wileyonlinelibrary.com]

To address this, in a critical first step, educational and training workshops, webinars, online classes and a literature review were organized. To minimize the technical variations, well-standardized EuroFlow-recommended next-generation flow methodologies were adopted as standard operating procedures with no modifications. EuroFlow-validated flow cytometer instruments were used. After the training phase, a total of 21 laboratories across Brazil submitted at least 10 BMRD FCS files for the initial phase, followed by centralized reanalysis by two experienced laboratories. In the sequential phase, laboratories further submitted BMRD FCS files. Such longitudinal evaluation strategies are crucial for real-world implementation of MFC-BMRD assays. This system identified systematic problems, pitfalls and suggested corrective actions. Importantly, it ensured uniformity of MRD interpretation. A high degree of concordance was observed in both phases of the study. Discordant results were noted at very low MRD levels (<0.01%) which is not entirely unexpected.

A real challenge with advancing technologies such as MFC-BMRD with high sensitivity is ensuring uniform implementation and interpretation across different centres. Successful multicentric standardization is the only way forward to take the technology to community level, ensuring maximum benefit. This is especially true in LMIC, where many laboratories with variable expertise may cater to a large number of patients. In addition to technical problems such as requirement of uniform processing, acquisition and analysis protocol, the logistic hurdles of multicentric laboratory-oriented collaborations can be prohibitive, including the fragile human ego. We can be only as strong as our weakest link! Moreover, single point evaluation is also not enough as it may not ensure longitudinal adherence to protocols over a period of time. This study provides a simple framework of establishing multicentric standardization of a BMRD assay with longitudinal evaluation ensuring uniform results across different laboratories. These results pave a path for more widespread utilization of highly sensitive BMRD technologies in the LMIC setting.

ORCID

Gaurav Chatterjee https://orcid.org/0000-0002-6505-7898
Nikhil Patkar https://orcid.org/0000-0001-9234-2857

REFERENCES

1. Ikoma-Colturato MRV, Bertolucci CM, Conti-Spilari JE, Oliveira E, Simioni AJ, Figueredo-Pontes LL, et al. Multicentric standardization of minimal/measurable residual disease in B-cell precursor acute lymphoblastic leukaemia using next-generation flow cytometry in a low/middle-level income country. Br J Haematol. 2023;200:381–4.
2. van Dongen JJM, van der Velden VHJ, Brüggemann M, Orfao A. Minimal residual disease diagnostics in acute lymphoblastic leukemia: need for sensitive, fast, and standardized technologies. Blood. 2015;125(26):3996–4009.
3. Dworzak MN, Gaipa G, Ratei R, Veltroni M, Schumich A, Maglia O, et al. Standardization of flow cytometric minimal residual disease evaluation in acute lymphoblastic leukemia: multicentric assessment is feasible. Cytometry B Clin Cytom. 2008;74B(6):331–40.
4. Julie I, Jenny J, Paul V, Marian C, Lynne M, Lisa E, et al. Establishment and validation of a standard protocol for the detection of minimal residual disease in B lineage childhood acute lymphoblastic leukemia by flow cytometry in a multi-center setting. Haematologica. 2009;94(6):870–4.

5. Borowitz MJ, Wood BL, Devidas M, Loh ML, Raetz EA, Salzer WL, et al. Prognostic significance of minimal residual disease in high risk B-ALL: a report from Children's oncology group study AALL0232. Blood. 2015;126(8):964–71.
6. Theunissen P, Mejstrikova E, Sedek L, van der Sluijs-Gelling AJ, Gaipa G, Bartels M, et al. Standardized flow cytometry for highly sensitive MRD measurements in B-cell acute lymphoblastic leukemia. Blood. 2017;129(3):347–57.
7. Tembhare PR, Subramanian Pg PG, Ghogale S, Chatterjee G, Patkar NV, Gupta A, et al. A high-sensitivity 10-color flow cytometric minimal residual disease assay in B-lymphoblastic leukemia/lymphoma can easily achieve the sensitivity of 2-in-106 and is superior to standard minimal residual disease assay: a study of 622 patients. Cytometry B Clin Cytom. 2020;98(1):57–67.
8. Chatterjee G, Sriram H, Ghogale S, Deshpande N, Khanka T, Girase K, et al. Mimics and artefacts of measurable residual disease in a highly sensitive multicolour flow cytometry assay for B-lymphoblastic leukaemia/lymphoma: critical consideration for analysis of measurable residual disease. Br J Haematol. 2022;196(2):374–9.
9. van Dongen JJM, Lhermitte L, Böttcher S, Almeida J, van der Velden VHJ, Flores-Montero J, et al. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. Leukemia. 2012;26(9):1908–75.
10. Ikoma-Colturato MRV, Beltrame MP, Furtado FM, Pimenta G, da Costa ES, Azambuja AP, et al. Minimal residual disease assessment in acute lymphoblastic leukemia by 4-color flow cytometry: recommendations from the MRD working Group of the Brazilian Society of bone marrow transplantation. Hematol Transfus Cell Ther. 2021;43(3):332–40.

How to cite this article: Chatterjee G, Patkar N. United we stand, divided we fall. Multicentre standardization of measurable residual disease assessment in acute leukaemia is the way forward. Br J Haematol. 2023;200(3):277–279. https://doi.org/10.1111/bjh.18533