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COMMENTARY

From gating to computational flow cytometry: Exploiting artificial intelligence for MRD diagnostics

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The era of AI-based methods to improve flow cytometry diagnostics in haematology is now at the beginning. The study by Nguyen and colleagues explored an emerging machine learning approach to assess phenotypic MRD in chronic lymphocytic leukaemia patients, showing that such AI-driven computational analysis may represent a robust and feasible tool for advanced diagnostics of haematological malignancies.

Commentary on: Nguyen et al. Computational flow cytometry provides accurate assessment of measurable residual disease in chronic lymphocytic leukaemia. Br J Haematol 2023 (Online ahead of print). doi: 10.1111/bjh.18802

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AI, CLL, flow cytometry, machine learning, MRD

In this issue, Nguyen et al.[1] describe the application of a promising approach of computer-assisted high-dimensional immunophenotype analysis – commonly known as *computational flow cytometry* (CFC) – for the assessment of minimal/measurable residual disease (MRD) in chronic lymphocytic leukaemia (CLL) patients. This study shows that such artificial intelligence (AI)-supported MRD diagnostics can be highly accurate and reproducible, as well as readily implementable in the routine workflow, compared with gold standard MRD expert analysis, using conventional manual gating procedures. Over the last decades, MRD eradication has progressively been recognized as a valuable prognostic factor, associated with improved clinical outcomes in virtually-all haematological malignancies, including CLL.[2] Recently, with the advent of effective 'fixed-duration' first-line treatments for CLL patients,[3,4] the undetectable MRD status ($<10^{-4}$) and different MRD kinetics (i.e. time to MRD conversion, to MRD doubling and to 10^{-2} MRD threshold) have emerged as pivotal parameters for therapeutic decision-making, mainly when choosing either to continue or to withdraw treatments in high-risk CLL patients.[5] In this clinical setting, the CFC method here reported can provide an impressive tool to perform consistent immunophenotypic identification, accurate quantification and insightful sub-classification of rare CLL clones, with potential benefit for the patients.[1]

From a methodological point of view, multiparametric flow cytometry (MFC) has essentially been nearer to 'old-fashioned cytomorphology',[6] rather than to molecular and genomic techniques, mainly because both classic microscopy and flow cytometry typically depend on individual operator's expert analysis of some discrete information (either optical or digital, respectively), about phenotypic features of normal and pathological cells. However, in the last years, advanced AI-based systems – coming from the research to routine clinical applications – have started to change this notion, by providing an unprecedented opportunity to automatically elaborate large amounts of multidimensional immunophenotypic data, acquired by last-generation high-throughput (≥8-colour) flow instruments (now commonly available for haematological diagnostics). At the core of this AI innovation, by applying machine learning models with specific clustering algorithms to wide cytofluorimetric datasets, new predictive models are independently generated, being directly learned from the acquired data, with no or minimal human interventions. Operatively, such computational tools can build a detailed reference map of the 'immunophenotypic landscape' (derived from multiparametric

profiling of surface antigens' expression at single-cell level), which is then exploited to provide a consistent measurement of distinct cell populations, detectable in the diagnostic samples. By comparison, standard MFC analysis is actually based on a single operator's manual immunological gating on sequential bidimensional plots; thus, it is intrinsically burdened by the dependence on time-consuming expert analysis, with well-known reproducibility problems (partially addressable by standardization of technical procedures and gating protocols) (Figure 1).

In this view, it is likely that AI-driven flow cytometry diagnostics may constitute a pivotal advance in this field, as providing a reliable and feasible, comprehensive (multidimensional) immunological analysis of the whole immunophenotypic dataset, with some evident advantages, and few limitations.[7] Basically, CFC clinical applications appear largely automatable (traditional gating process may still serve for additional expert validation), time-efficient (with an average run time of few seconds/minutes per sample), reproducible and potentially harmonizable across laboratories. In addition, CFC can help to extend our clinico-biological knowledge about relevant disease immunophenotypes, possibly endowed with prognostic significance, in different haematological malignancies. As a reasonable drawback, the implementation of sophisticated CFC methods in routine haematology laboratories could raise an issue about general feasibility. However, it seems the case that, while the computational development of new CFC algorithms typically requires the specific expertise of computational biologists – called to team up with flow cytometrists and clinical haematologists – eventually, the ultimate routine implementations of CFC analysis should be much more 'user-friendly', possibly well supported by software dedicated to end-users.

Of course, to date, CFC methods still need to be validated in large multicentric studies, and the clinical use of such AI-based systems should properly be regulated and harmonized by shared recommendations among worldwide laboratories, aiming to provide reliable and transparent diagnostic tools. So far, different machine learning models have successfully been applied to elaborate flow cytometric data, either by using unsupervised methods (e.g. FlowSOM in the setting of AML diagnosis),[8] or with supervised/semi-supervised techniques (e.g. deep neural network for CLL MRD detection).[9] In their work, Nguyen and colleagues[1] developed and tested an original application of FlowSOM for the assessment of CLL MRD in peripheral blood and bone marrow samples, compared with standard (human) expert analysis. By using training datasets, this unsupervised hierarchical clustering method generated a self-organizing map (SOM) with several nodes, outlining the full immunophenotypic landscape of normal and pathologic B-cell populations. Then, the reference SOM was used to automatedly detect MRD cells in three large validation cohorts of CLL patients.[1]

Flow cytometry experts and clinical haematologists are now called to become familiar with general concepts of computational analysis applied to routine diagnostics,

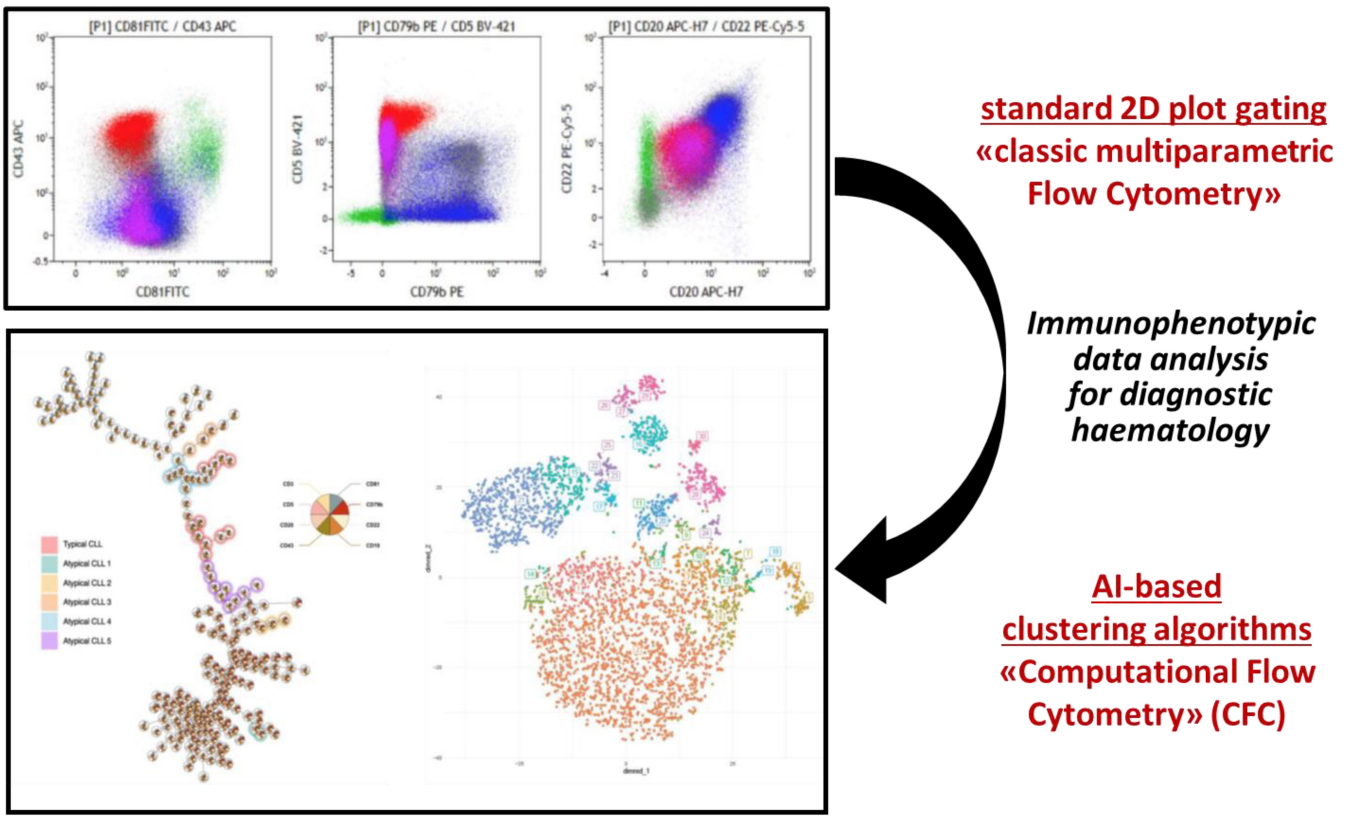

FIGURE 1 Evolution of immunophenotypic data analysis (2D plots, top; MST, bottom left; t-SNE, bottom right; all graphs from Nguyen et al.[1]).

to correctly set up such novel diagnostic methodologies in the laboratory workflow, as well as to guide the development of this expanding area of AI-driven clinical research. Of note, automated clustering systems, combined with n-dimensional immunophenotyping techniques, may allow to disclose new putative cell subpopulations, at high-resolution level (beyond 1 cell out of 10^4). Some of these novel phenotypic subtypes may be associated with unknown biological significance, which, in turn, could display a prognostic value in specific disease settings. For instance, in the work by Nguyen et al.,[1] several atypical disease subtypes (named from CLL-1 to CLL-5) have been identified, potentially waiting for further clinico-biological investigations.

In perspectives, AI-based methods clearly offer the most effective way to elaborate and usefully interpret the large-scale datasets provided by the latest diagnostic technologies, in different fields of haematological diagnostics.[10] In addition to flow cytometry, also when applied to NGS data, neural networks and other machine learning algorithms have yielded new insights on tumor heterogeneity and clinico-biological classifications. Similarly, modern cytomorphology is evolving towards an AI-assisted 'digital microscopy', allowing automated pattern recognition and classification of acquired cell images. New opportunities and new challenges, including procedural and ethical caveats, are coming with the diffusion of AI-driven diagnostic methods in haematology, paving the way towards highly personalized therapeutic management of haematological patients.

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