Automated Analysis of Clinical Flow Cytometry Data

A Chronic Lymphocytic Leukemia Illustration

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KEYWORDS

• Chronic lymphocytic leukemia • Minimal residual disease • Cell-based diagnostics • Automated gating • Cluster analysis • Flow cytometry • FLOCK

KEY POINTS

- Traditional manual gating analysis of cytometry data cannot effectively address the scale and complexity of data generation from modern cytometry instrumentation.
- Bioinformatics investigators have developed a collection of computational methods for automated identification of cell populations from high-dimensional flow cytometry data; a small subset of these methods has been evaluated for their use in diagnostics applications of leukemia and lymphoma with promising results.
- By applying computational pipelines to classify and compare chronic lymphocytic leukemia (CLL) samples with healthy controls, the pilot study reported in this article illustrates the use of these methods to determine that traditional CLL definition based on CD5 and CD19 alone can be improved by also examining the expression levels of CD10 and CD79b in an automated fashion.
- Clinical validation of these computational approaches is ongoing and essential to realize the true potential of these methods for use in the clinical diagnostic laboratory.

BACKGROUND

Cells of the peripheral blood can serve as sentinels of the physiologic and pathologic state of an organism. Normal vascular recirculation and extravascular migration allow these cells to touch every part of the body. The number and phenotype of blood cells

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are also constantly influenced by the sea of cytokines, growth factors, hormones, and other small molecules they are bathed in, such that the cellular constituents of blood also reflect its molecular constituents. Thus, a detailed, accurate, and consistent representation of the qualitative and quantitative properties of blood cells can be used to understand the mechanistic underpinnings of disease and to identify potential biomarkers of disease diagnosis, prognosis, and therapeutic response.

In the 1960s, Alexander Vastem recognized that the enumeration of blood cell types (complete blood count, CBC) could be used diagnostically as evidence for certain kinds of infections and malignancies. Although the CBC has emerged as a critical laboratory assay, it cannot detect, with any detail, the phenotypes of the enumerated blood cells. Flow cytometry (FCM) represents an advancement in the analysis and characterization of blood cells, enabling researchers and clinicians to identify the surface antigen expression of blood cells using fluorochrome-conjugated antibodies, multiple lasers to provide specific excitation of fluorochromes, and several detectors to quantitate the emitted fluorescent signal. Indeed, using a simple cocktail of antibodies, each conjugated to defined fluorochromes with known emission ranges, an investigator can identify specific lymphoid and myeloid populations in peripheral blood, as well as the expression of antigenic determinants, some of which can be diagnostic or prognostic.

In 2007, Davis and colleagues[1] published recommendations from an expert panel regarding the medical indications for performing FCM-based diagnostic testing, including cytopenias, elevated leukocyte counts, atypical cells in bodily fluids, plasmacytosis or monoclonal gammopathy, and organomegaly. Based on the individual indications, specific staining panels are selected to target the likely cell suspects. For example, at the University of California, San Diego (UCSD) Center for Advanced Laboratory Medicine (CALM), 10 different tubes/panels are in routine use to aid in the diagnosis of acute and chronic leukemia. **Table 1** shows an example of 3 such tubes, the fluorescent channels used, and the antigens detected.

Although the use of these panels has demonstrated accurate and clinically actionable diagnosis and classification of hematolymphoid neoplasms, there is room for improvement, given the genetic and phenotypic diversity of blood cell diseases. For example, although flow cytometry can accurately diagnose acute promyelocytic leukemia (APL) based on multiple surface antigens, molecular subclassification of APL using cytogenetic detection of the t(15;17) translocation is diagnostic for a subtype that responds to all trans-retinoic acid. The fact that this leukemia appears to be derived from a distinct population of immature granulocytes suggests that this important subclassification could potentially be achieved using more complex staining panels alone without the need for cytogenetics. However, despite the routine use of complex (ie, >8 color) staining panels in research laboratories in recent years, high-complexity FCM panels have not been incorporated into routine use in the clinical FCM laboratory. In part, the lack of consensus of diagnostically relevant antigens and

Table 1
Markers used for diagnosis of acute and chronic leukemia

	Acute Myeloid Leukemia Panel					Chronic Lymphocytic Leukemia Panel			
	FL1	FL2	FL3	FL4		FL1	FL2	FL3	FL4
Tube 1	CD15	CD33	CD45	CD34	Tube 1	CD45	CD5	CD3	CD19
Tube 2	CD2	CD117	CD45	CD34	Tube 2	CD43	CD79a	CD5	CD19
Tube 3	HLA-DR	CD7	CD13	CD34	Tube 3	CD20	CD38	CD5	CD19