Policy | Brief

Flow cytometry for the diagnosis of acute leukemia in Ethiopia

Summary

Flow cytometric phenotypic has become one of the principal means of diagnosing acute leukemia, and is routine in many western settings to guide patient management. The Armauer Hansen Research Institute has over 30 years of experience in flow cytometry, and has conducted several research pilot studies into its use for leukemia diagnosis in the Ethiopian setting. Our findings confirm results of many previous clinical studies and have demonstrated the feasibility of this approach for many patients and the hematologists and pathologists at Black Lion hospital are enthusiastic about its application; however, the cost and technical and interpretative skill required is not trivial.

We propose a two-phase plan of implementation of flow cytometry phenotyping. The first phase will last two years and focus on intensive training and gradual roll out with comprehensive monitoring at Black Lion hospital. After review of strengths and weakness of the program, a second phase will be entertained, in which appropriate modifications will be introduced, including expansion of involved sites to peripheral oncology centers and upgrade of flow cytometers.

Brief background of flow cytometry

Acute leukemia, comprised generally of acute lymphocytic and acute myelogenous leukemia is one of the leading causes of cancer in Ethiopia and a common cause of hospital admissions (1). Diagnosis has relied historically on evaluation of cell morphology of peripheral blood smears or bone marrow biopsies, which describe general cellular features and represent a good low-cost starting point for diagnosis. However, with time it has become clear that there exist many subtypes of ALL and AML, and that different therapies are optimal for different leukemia subtypes. More recently, several approaches have been utilized to increase the diagnostic resolution of acute leukemia. These include flow cytometric phenotyping (2-5), as well as several methods

to characterize genetic or chromosomal abnormalities related to the malignant process and which can typify certain leukemias (6). In Ethiopia, leukemia diagnosis has always been based exclusively on clinical findings as well as cell morphological approaches, and the availability of improved adjunct methods for diagnosis are needed to guide optimal medical care of leukemia patients (7).

At AHRI, we are currently evaluating multiple approaches; however, the focus of this policy brief is on flow cytometric phenotyping. The focus on flow cytometry is that it represents a methodology which AHRI has many years of experience with; moreover, as part of the ART HIV program, a large number of FACScalibur flow cytometers in the country utilized as part of the CD4 count analysis. The availability of such cytometers allows the development of a common platform which can be utilized both in the large referral center at Black Lion Hospital, as well as in other labs around the country and multiple regional oncology centers throughout Ethiopia.

Leukemic cells express a large number of molecules on the cell surface and in the cytoplasm. All of these molecules are expressed by normal leukocytes, but to varying degrees, such that a large number of subsets of leukocytes can be defined with unique patterns of molecular expression, and these in turn are associated with different functional properties. Leukemia cells can be considered as belong to a unique subset of leukocytes, but unlike normal cells have developed into a malignancy. Many leukemia expresses the pattern of molecules expressed by normal leukocytes, but typically have some abnormal expression patterns as well. The pattern of molecule expression can typically (but not always) discriminate between different types of leukemia cells and normal cells. Flow cytometry has thus become a widely used adjunct procedure for leukemia diagnosis globally (4).

In simple terms, blood cells are stained with reagents (monoclonal antibodies) specific to the cell molecules of interest. The cells are aspirated into the flow cytometer and pass through a series of laser beams which result in the detection of signals related to the numbers of each specific molecule of interest on each cell. Flow cytometers can simultaneously measure multiple molecules. The expression of the molecules is depicted in a series of plots which are later analyzed and integrated, yielding a likely diagnosis.

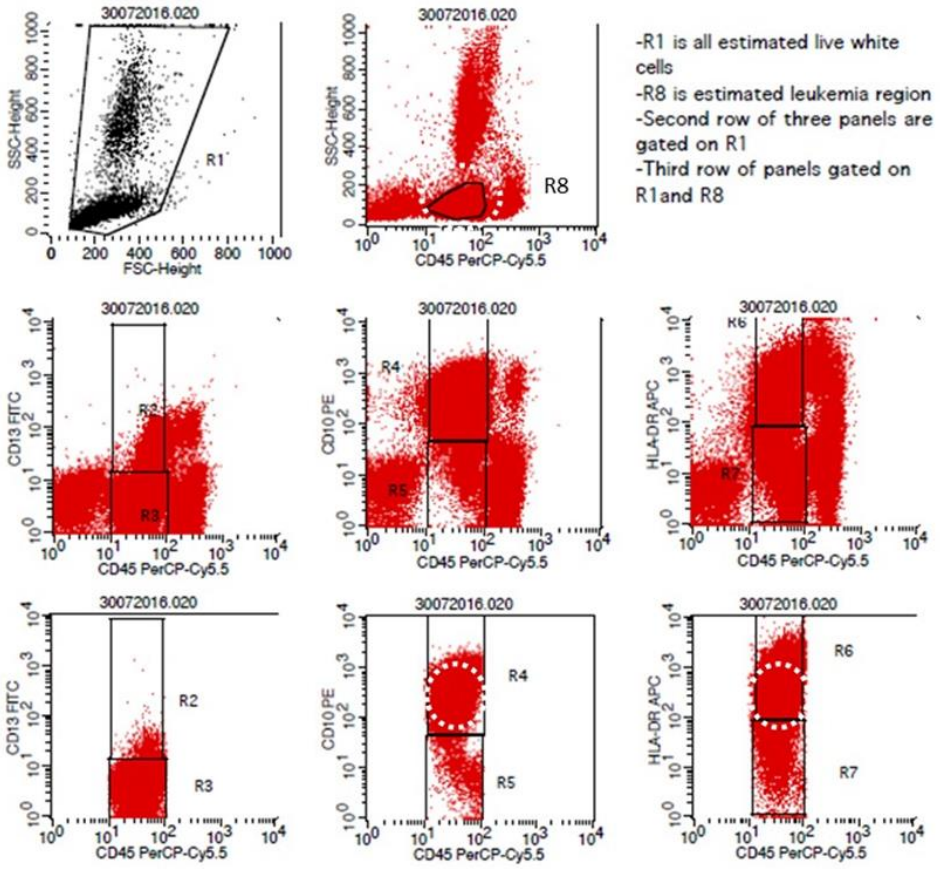

Figure 1. A representative set of plots involved in flow cyometric phenotyping. Leukemia cells are identified in the encircled region of the upper right-hand plot, expressing intermediate levels of CD45 and low side scatter (SSC). Such leukemia cells are further selected and depicted for the expression of other molecules shown in the bottom row. Thus these leukemia cells expressed low CD13 (lower left), but high CD10 (lower middle) and high HLA-DR (lower right). Typically many more surface molecules are utilized to define the final phenotype used for diagnosis.

Important Findings

- We have demonstrated the feasibility of flow cytometry within the Ethiopian setting, having completed two research studies with two more ongoing (8). In the two studies completed we found the degree of concordance between diagnoses determined by flow cytometric phenotyping and cell morphology is about 80%, which is typical of many such research comparisons. Flow cytometry was shown to be particularly good at discriminating differences between relatively undifferentiated subtypes of AML and ALL. For example, instances were found in our studies where patients were diagnosed by morphology as AML, but responded poorly to therapy, returned to the clinic and found flow cytometry to have T-ALL, indicating an alternative therapy.

- The training needed is intensive. We developed multiple introductory training modules, and provided introductory background to multiple lab staff at Black Lion, and also provided a course to a group of pathologists on interpretative skills needed for flow-based diagnosis.

- For their project, the involved MSc and PhD students have received at least 40 hours in-person instrument training to gain adequate competency.

- We have also developed a number of software tools to be used in conjunction with currently commercially available software, to improve quality control and efficiency, and have also prepared a customized database repository to house all patient flow image data, a valuable resource for referral and future training purposes.

Policy Implications

- The implications of our research are that flow cytometry does offer an improvement in leukemia diagnosis and hence optimal patient management. Importantly, the hematologist clinicians at Black Lion, currently the major diagnosis and treatment center are very much in favor of continued use of flow-based approaches. The method is both labor and skill intensive, and any program implemented at a national or regional level will require intensive training and ongoing monitoring.

Policy/Intervention

We propose a gradual implementation program in phases.

- The first phase will last two years, and will focus on establishing the technique in full time use at Black Lion hospital, with oversight and monitoring by AHRI to ensure quality control and competency. This will entail continued training of Black Lion laboratory staff, including those with post-graduate degrees, as well as pathologists.

- We will use the prepared training modules as well as the many analytical applications to improve quality, efficiency and monitoring. In addition, we will conduct clinical research to quantify the impact of flow cytometry on patient outcomes. All data will be placed into a data repository, with review of all patient records by AHRI as well as selected records from internationally experienced clinical pathologists from abroad.

- The primary cost of phase one is approximately 30,000 USD total for two years. This primarily covers the costs of needed reagents, but will also cover expenses for periodic workshops. Additional training costs will be covered by AHRI. During phase I, AHRI will be separately funding and conducting pilot studies in peripheral oncology centers in the country such as Jimma University to evaluate feasibility in peripheral settings as well. At the end of phase I, we will re-evaluate the program from a need and cost-effectiveness perspective, and in collaboration with the Ministry of Health make decisions regarding a) maintenance of the program at Black Lion hospital, b) expansion of the program to other regional oncology centers nation-wide, c) consideration of costs of flow cytometry upgrades (machines typically cost 100,000-150,000 USD).

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