CR

CASE REPORT

E.P.C.M.S.

Case Studies with Flow Cytometry for Leukemia Diagnosis

Kantibhai M. Patel, M.Ed, I (ASCP), CHS (ABHI)
Delfina C. Dominguez, Ph.D., MT (ASCP)
Harry Wilson, M.D.

INTRODUCTION:
Advances in instrumentation and technology such as development of monoclonal antibodies, improvement in fluorescent dyes, sophisticated data analysis, and improved software, have led to the development of flow cytometry (11).The ability to examine thousands of cells in a very short period of time makes flow cytometry a very powerful tool. As a result, flow cytometry immunophenotyping is now an essential component in the classification of leukemia and lymphoma (3, 8, 12). In this report, we present two cases of leukemia to demonstrate the utility of flow cytometry in clinical practice

BACKGROUND AND SIGNIFICANCE:
Immunophenotyping by flow cytometry in conjunction with morphology and other pertinent clinical information is used to help with the diagnosis and classification of hematologic disorders, especially leukemia and lymphoma (5). The principal goal of immunophenotyping is to aid with determination of cell lineage, e.g. myeloid versus lymphoid leukemia, and T versus B cell lymphocytic lymphoma. In addition, due to its high sensitivity, flow cytometry is helpful in detecting minimal residual disease following treatment, as well as with early relapse (6, 7, 13). It is also used to monitor patients on immunosuppressive therapy following transplantation (10).

Cell surface immunophenotyping can be performed on peripheral blood, bone marrow, or lymph node tissue. It involves incubating the patient's specimen with monoclonal antibodies that have been conjugated to a fluorescent dye. Cluster differentiation (CD) describes a cluster of antibodies that identify cell surface or intracytoplasmic antigens (6). The flow cytometer uses a laminar flow to pass the single cell stream through a laser light beam to excite the fluorescent probes on the cells. Then various detectors collect the photons, which are converted into electrical signals by the photomultiplier tubes (PMT) and fed into a computer to analyze various cell populations of interest (1, 2, 6)

There are two schools of thought regarding the determination of leukemia and lymphoma panels. Some laboratories run panels encompassing a large number of antibodies on every sample. This is time consuming and expensive, and although it does provide a large amount of information, some may not be directly relevant to the clinical context. Other laboratories use a quick sample preparation method and run a screening panel which includes markers of interest for specific hematologic diseases (3, 4, 12). Once this information is obtained, a follow-up panel using additional markers can be run if needed. This practice, while also comprehensive, can result in a delay in data acquisition. Our laboratory prefers a moderate comprehensive panel derived at evaluating specific diagnosis, a compromise between these two approaches.

CASE REPORT # 1 Chronic Lymphocytic Leukemia
Case Presentation:
A 72-year-old female with a left kidney mass underwent fine needle aspirate biopsy which provided a diagnosis of renal cell carcinoma. A left nephrectomy followed verifying a clear cell renal cell carcinoma, nuclear grade 3. Subsequent peripheral blood evaluation raised a suspicion of possible abnormal white cells in her peripheral blood.

Peripheral blood (Figure 1) showed anemia with hemoglobin 9.5 gm/dl and hematocrit 28%. Red cells showed mild anisocytosis. Platelets were adequate at 185K/MM3. White cells were increased with a total white count of 22 K/MM3. There was a mild neutrophilic leukocytosis (ANC=9K/MM3) with an abnormal lymphocytosis (ALC=12K/MM3). Granulocytes showed the usual features of maturation with occasional band forms. Lymphoid cells were of intermediate size and showed a somewhat monotonous mildly activated cytomorphology. The increased lymphoid cells raised a suspicion of possible lymphoproliferative disorder. Monocytes were mildly increased (AMC=1K/MM3) and also showed features of activation. Occasional smudge cells were seen.

A flow cytometric immunophenotype studies were performed (Figure 2,3,4) on the peripheral blood derived white cells with the following results:
Granulocytes: 50% (CD13/CD33 positive) ; Monocytes: 5% (CD11c/CD14 positive) ; Mature T–cell lymphocytes: 3% (CD2/CD3/CD5 positive with a CD4:CD8 T-cell ratio of 2:1). Mature B–cell lymphocytes: 0%; Abnormal B lymphoid cells: 25% (B-cells positive for CD5, CD19, CD20, weak CD22, HLA-DR, and CD45 with lambda surface light chain monoclonality).

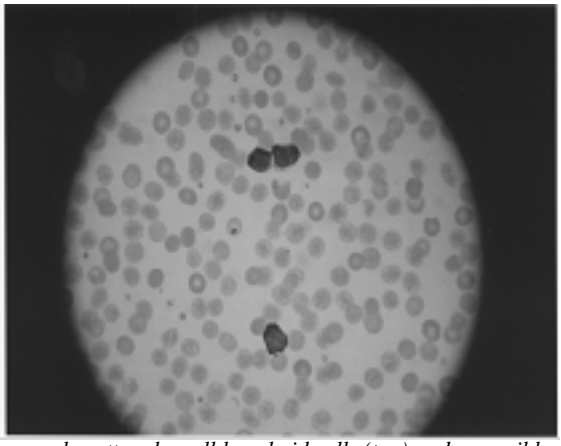
These immunophenotypic findings indicated a diagnosis of an early B-cell chronic lymphocytic leukemia in this patient with a known renal cell carcinoma. A confirmatory bone marrow examination with cytogenetics analysis was requested.

Continued on page 24

CR
CASE REPORT

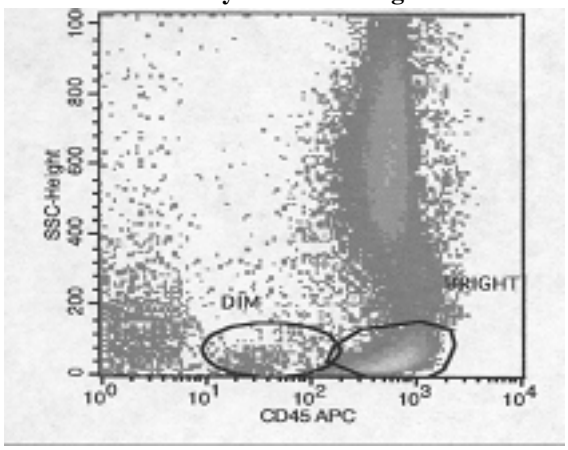
Case Studies with Flow Cytometry for Leukemia Diagnosis (Continued)

Figure 1. Peripheral blood microscopy:



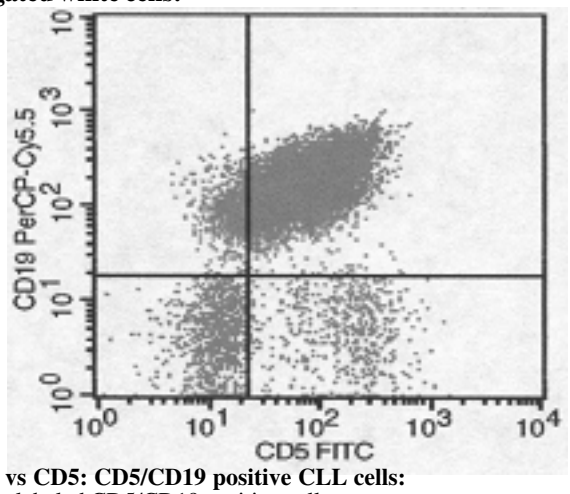
Two abnormal scattered small lymphoid cells (top) and a possible smudge cell (bottom) in this patient's peripheral blood consist with an early B-cell CLL.

Figure 2. Relevant Flow Cytometric Histograms:



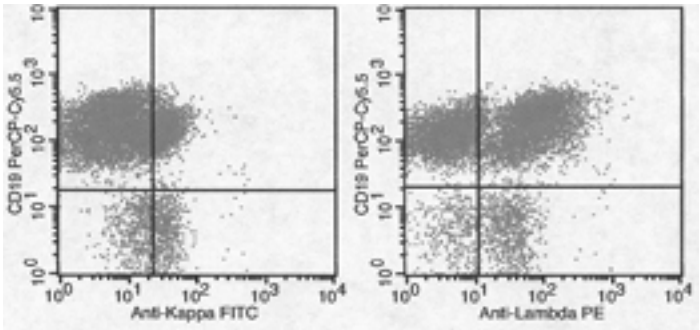
SSC vs CD45: CD45 is strong positive
Initial white cell identification histogram, with gated small cell CD45 positive lymphocytes; SSC = Side Scatter as the indicator of cytoplasmic complexity. CD45 = common leukocyte antigen marker.

Figure 3. Histograms obtained from the gated lymphocyte (small cell) gated white cells:



CD19 vs CD5: CD5/CD19 positive CLL cells:
Double labeled CD5/CD19 positive cells

Figure 4. CD19 vs kappa/lambda: Moderate lambda surface light chain positive and kappa negative CLL cells.



Positive double labeled CD19/lambda lymphoid cells (neoplastic population).

Summary of histogram panel results:

Markers:	CD10	CD19	CD20	CD22	CD38	CD45	HLA-DR	KAPPA	LAMBDA
CLL cells:	+	+	+	wk+	+	+	+	-	+

Final Diagnosis:
Peripheral blood showing anemia, mild neutrophilic leukocytosis, and a malignant lymphocytosis (12K/MM3) with abnormal B lymphoid cells; findings indicating an early B-cell chronic lymphogenous leukemia with monoclonal lambda light chain a CD5/CD19 double label positive immunophenotype. A confirmatory bone marrow examination was recommended.

Comment:
CLL is usually not discovered and diagnosed until there is significant lymphocytosis in the peripheral blood. However, by use of flow cytometry in this patient, a diagnosis was suggested at an earlier point in the patient's disease.

CASE REPORT # 2 Acute Lymphocytic Leukemia
Case Presentation:
A 4-year-old boy presented with a clinical history of suspected leukemia. Abnormal white cells were detected on routine peripheral blood examination for evaluation of leg pain.

Peripheral blood (Figure 5) showed anemia with hemoglobin 10.0 gm/dl and hematocrit 29%. Occasional ovalocytes were seen and a rare fragmented red cell was noted. The reticulocyte count was 1.2%. Platelets were adequate at 154K/MM3. White cells were increased with a total white cell of 11K/MM3. There was mild lymphocytosis with an ALC of 10K/MM3. Some atypical and immature lymphoid cells were seen with lymphoblasts identified at approximately 20% of the counted white cell components. There was mild neutropenia with an ANC of 1K/MM3.

Bone marrow examination showed hypercellular (98%) homogenous marrow white cells, with a marked replacement of usual hematopoietic elements by acute lymphogenous leukemia lymphoblasts. Scattered megakaryocytes were recognized. Other normal hematopoietic elements were sparse. The acute leukemic lymphoblasts were present at approximately 85% of counted components. Granulo-

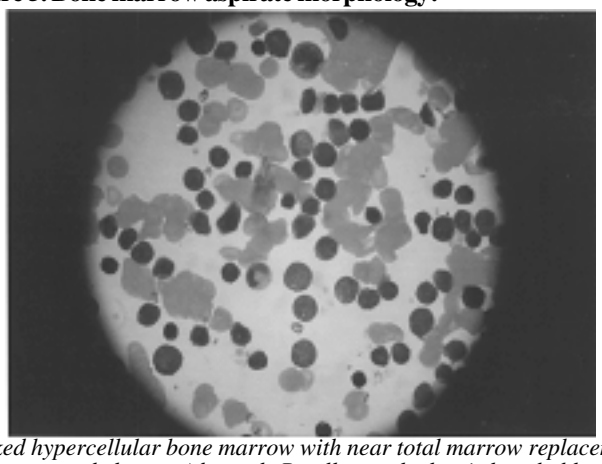
Continued on page 25

cytic forms were identified at 10% as noted by choractate esterase, Sudan black, and myeloperoxidase special stains. The acute leukemic lymphoblasts showed a mixed FAB L1 and L2 cytomorphology.

Flow cytometric immunophenotype studies (Figure 6,7) were performed on the bone marrow derived white cells from this patient with results as follows:
Granulocytes: 20% (CD13/CD33 positive) ; Monocytes: 1% (CD11c/CD14 positive)
Mature T–cell lymphocytes: 10% (CD5/CD7 positive with a reversed CD4:CD8 T-cell ratio of 0.6:1) Mature B–cell lymphocytes: 5% (polyclonal surface light chain staining); ALL Lymphoblasts: 50% positive CD10, CD19, CD34, CD38, HLA-DR stains; weakly positive CD22, CD45 stains; negative CD11c, CD20, CD61, and kappa/lambda surface light chain stains.

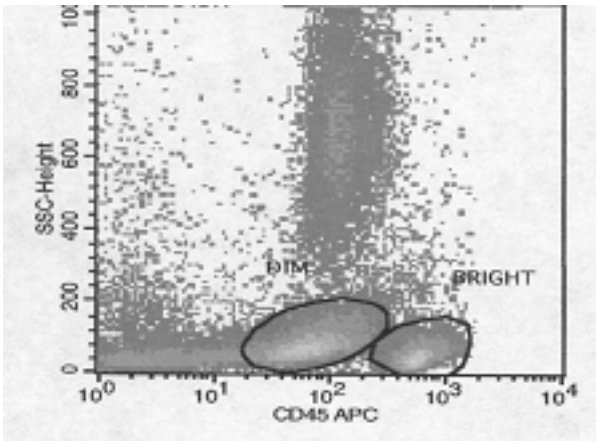
These flow cytometeric immunophenotype studies confirmed the morphologic impression of new onset acute lymphogenous leukemia, early B-cell type. A separate negative cyto-Mu study confirmed a precurcusor B–cell cytometric identity.

Figure 5. Bone marrow aspirate morphology:



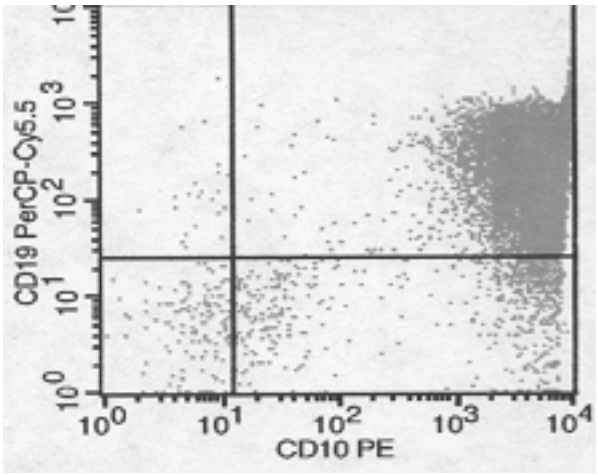
Marked hypercellular bone marrow with near total marrow replacement (sparce normal elements) by early B-cell acute leukemic lymphoblasts

Figure 6. Relevant Flow Cytometric Histograms:

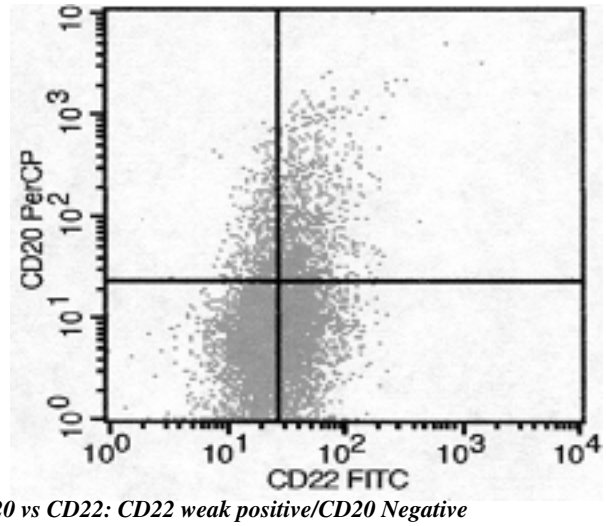


SSC vs CD45: CD45 is weak positive in the abnormal cells: *Initial white cell identification histogram with gated weak CD45 positive abnormal white (lymphocyte) cell population SSC = Side Scatter as the indicator of cytoplasmic complexity. CD45 = common leukocyte antigen marker*

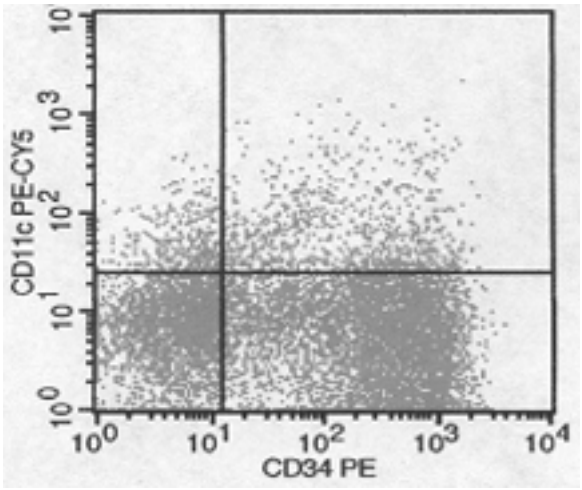
Figure 7. Histograms obtained from the abnormal cells (weak CD45) gated white cells:



CD19 vs CD10:CD10 strong positive/CD19 positive:
Double labeled CD10/CD19 positive cells for the abnormal white cell gate



CD20 vs CD22: CD22 weak positive/CD20 Negative



CD11c vs CD34:CD34 strong positive/CD11c negative

Continued on page 26

Summary of histogram results:

Markers:	CD10	CD11c	CD13	CD19	CD20	CD22	CD34	CD38	HLA-DR	CD45	CD61
ALL cells:	+	—	wk+	+	—	wk+	+	+	+	wk	+ —

Final Diagnosis:
Peripheral blood showing anemia, mild neutropenia, and a malignant lymphocytosis (10K/MM3) from a new onset precursor B-cell acute lymphogenous leukemia. Hypercellular (98%) bone marrow aspirate, clot, and biopsy preparations showing approximately 85% marrow involvement by acute lymphocytic leukemia with admixed FAB L1 and L2 lymphoblasts CD10/CD19/CD34 positive; findings diagnostic of new onset acute lymphocytic leukemia, precursor B-cell type.

Comment:
The bone marrow findings in this case were diagnostic for ALL. An immunologic subtype identification for ALL usually requires a flow cytometric immunophenotypic analysis. Indeed, if flow cytometry had been performed on the peripheral blood white cells from this patient, it probably would have detected ALL cells even sooner, without having to wait, as in this case, until abnormal peripheral cytopenias and clinical symptoms directed a bone marrow examination for diagnosis.

CONCLUSION:
Immunophenotypic findings in the first case provided an early diagnosis of early B-cell chronic lymphocytic leukemia (CLL) for a patient with known renal cell carcinoma. Bone marrow examination with cytogenetics analysis can further add in a specific subtype diagnosis. In the second case immunological identification of acute lympocytic leukemia (ALL) was made only possible with a flow cytometric analysis. Such definitive diagnosis could not have been accomplished without flow cytometric characterization of the malignant cells involved. These two cases illustrate the fact that flow cytometry can be a powerful tool for immune and hematopoietic cell identification. The amount of patient sample required is small and the procedure provides both sensitive and specific data for oncology diagnostics. Availability of such an instrument in El Paso allows for same day results with prompt initiation of treatment; in addition, flow cytometry facilitates the monitoring of therapeutic response in these patients.

Acknowledgements:
The cases were obtained from the 2006 pathology files of Sierra Providence Health Network. We would like to thank Judith Pester, MD, Arturo Vargus, MD, Anju Mani, MD, Gregory Elkins, MD, and Mrs. Mary Ann Pavia for their encouragement and support, and Mr. Everick Austin, Mrs. Delores C. Gonzalez, and Miss. Margarita Pena for their excellent technical assistance.

RERENCES
1. Becton Dickinson (BD) Biosciences, FACSCalibur System Users Guide, August, 1996, Becton Dickinson Immunocytometry Systems, San Jose, CA.

2. Becton Dickinson (BD) Biosciences, Cell Quest Users Guide, July, 2002, Becton Dickinson Immunocytometry Systems, San Jose, CA.

3. Braylan RC, Orfao A, Borowitz MJ, and Davis BH; Optimal Number of Reagents Required to Evaluate Hematolymphoid Neoplasias: Results of an International Consensus Meeting. In: Cytometry (Communications in Clinical Cytometry): 2001; 46:23-27.

4. Caldwell CW and Lacombe F. Evaluation of Peripheral Blood Lymphocytosis, Academic Information Systems, Inc. 2000.

5. Illoh OC; Current Applications of Flow Cytometry in the Diagnosis of Primary Immunodeficiency Diseases. Arch Pathol Lab Med., January 2004; 128:23-31.

6. Keren DF, McCoy Jr. JP, Carey JL. Flow Cytometry in Clinical Diagnosis: ASCP Press. 2001.

7. Leffell MS, Donnenber AD, and Rose NR: Handbook of human immunology: Clinical Application of Flow Cytometry, CRC press, Boca Raton, NY.1997; 381-476.

8. Morse EE, Yamase HT, Greenberg BR, Sporn J, Harshaw SA, Kiraly, Ziemba RA, and Fallon MA; The role of flow Cytometry in the diagnosis of lymphoma: a critical analysis. Annals of Clinical and Laboratory Science, 1994; 24:1, 6-11.

9. Nguyen D, Diamond LW, and Braylan RC; Flow Cytometry in Hematopathology A Visual Approach to Data Analysis and Interpretation. Humana Press. 2003.

10. Parker JW; Flow Cytometry in the diagnosis in hematologic diseases, Annals of Clinical and Laboratory Science, 1998; 16:6, 427-442.

11. Rosen, S; Flow Cytometry: an underexploited diagnostic power: Medical Laboratory Observer, March, 1998.

12. Stewart CC, Behm FG, Carey JL, Cornbleet J, Duque RE, Hudnall SD, Hurtubise PE, Loken M, Tubbs RR and Wormsley S: U.S.-Canadian Consensus Recommendations on the Immunophenotypic Analysis of Hematologic Neoplasia by Flow Cytometry: Selection of Antibody Combinations. In Cytometry (Communications in Clinical Cytometry).1997; 30:231-235

13. Stewart CC and Nicholson KA. (Edited) Immunophenotyping, Wiley-Liss, New York, NY. 2000.

14. Sun T, Sangline R, Ryder J, Gibbens K, Rollo C, Stewart S and Rajagopalan C; Gating Strategy for Immunophenotyping of Leukemia and Lymphoma, American Journal of Clinical Pathology, 1997;108:152-157.

Kantibhai M. Patel, M.Ed, I (ASCP), CHS (ABHI), Ph.D. Student, University of Texas at El Paso, College of Health Sciences; Supervisor, Transplant Immunology Laboratory, Sierra Medical Center, El Paso, Texas.

Delfina C. Dominguez, Ph.D., MT (ASCP), Associate Professor, University of Texas at El Paso, Texas, College of Health Sciences, El Paso, Texas.

Harry Wilson, M.D., Pathologist, Providence Memorial Hospital, El Paso, Texas.