Flow Cytometric Diagnosis of Low Grade B-cell Leukemia/Lymphoma

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Flow Cytometric Analysis of Hematolymphoid Neoplasia

- Standard of care—not research
- Guidelines available for medical indications
- Plays a vital role in diagnosis and sub-classification

Flow Cytometric Diagnosis of Chronic Lymphocytic Leukemia (CLL)

- CLL is the most common leukemia in the western world
- Diagnosis is routinely made by a combination of flow cytometry, WBC and review of clinical history
- Flow cytometric diagnosis is accepted medical practice
Chronic Lymphocytic Leukemia (CLL)

CLL Incidence: by country

![Bar chart showing CLL incidence by country](chart.png)

- Australia
- USA
- Ireland
- Italy
- Switzerland
- Canada
- France
- Austria
- Czech Republic
- Denmark
- Spain
- UK
- Germany
- Poland
- Sweden
- the Netherlands
- Brazil
- Costa Rica
- Japan
- China
- Ecuador

Legend:
- **Male**
- **Female**

Incidence rate per 100,000
Chronic Lymphocytic Leukemia (CLL)

Guidelines for the Diagnosis and Treatment of Chronic Lymphocytic Leukemia: A Report from the International Workshop on Chronic Lymphocytic Leukemia (IWCLL) updating the National Cancer Institute-Working Group (NCI-WG) 1996 guidelines:

The diagnosis of CLL requires the presence of more than or equal to $5 \times 10^9/L$ B lymphocytes (5000/µL) in the peripheral blood for the duration of at least 3 months.

Clinical history is relevant

Blood. 2008;111:5446-5456)
Flow Cytometric Diagnosis of CLL:

If detect by flow cytometry the presence of monoclonal B-cells with appropriate immunophenotype in the peripheral blood and at least $5 \times 10^9$/L B lymphocytes (5000/µL)* - Diagnosis is CLL

If less than $5 \times 10^9$/L B lymphocytes (5000/µL) - **Diagnosis is Monoclonal B-Cell Lymphocytosis**

- Absolute lymphocyte count elevated: Monoclonal lymphocytosis with clinical lymphocytosis:
- Absolute lymphocyte count not elevated: Low count monoclonal lymphocytosis

* duration of at least 3 months

Clinical history and WBC results relevant
Flow Cytometric Diagnosis of CLL: Not a Chemistry Test But a Clinical Diagnosis

54% B-cells
HCL

54% B-cells
CLL

48% B-cells
Polyclonal

46% B-cells
Polyclonal

Need more than a B-cell lymphocytosis for Diagnosis of CLL
Differential Diagnosis in CLL:

- Mantel Cell lymphoma
- Splenic Lymphoma with Villous Lymphocytes (SLVL)
- Lymphoplasmacytic lymphoma
- Follicular Lymphoma
- PLL
- HCL
- And if not determining lymphocytosis is B-cell-other non-Hodgkin’s lymphomas, including T-cell.
Flow Cytometric Diagnosis of CLL: Not a Chemistry Test But a Clinical Diagnosis

Can we just count CD5+ B-cells?

**CLL**

**MCL**

**HCL**

**Polyclonal**

**SF13 211 pb_08_B-5 new.fcs**

**SF12 324 pb_08_B-6+ K-p APC.fcs**

**SF13 228 pb_08_B-5 new.fcs**

**SF12 904 pb_08_B-6+ K-p.fcs**

**SF12 904 pb_08_B-6+ K-p.fcs**

**SF12 904 pb_08_B-6+ K-p.fcs**

**SF12 904 pb_08_B-6+ K-p.fcs**
Flow Cytometric Diagnosis of CLL: Similar to Looking at H&E Slide

CLL with vaguely nodular growth centers

Follicular Lymphoma

Reactive
Flow Cytometric Diagnosis of CLL: Appropriate Immunophenotype

- **CD19+CD5+**
- **CD23**
- Dim to negative CD81
- Dim CD20, dim CD22
- Dim to negative CD79b
- Monoclonal

**CLL**
Flow Cytometric Diagnosis of CLL: Appropriate Immunophenotype

CD19+CD5+
Dim Partial CD23+
Moderate CD81
Moderate CD20&CD22
Moderate CD79b
Monoclonal

MCL
CLL Diagnosis is Routinely Performed by Flow Cytometry:

- $5 \times 10^9$/L B-cells (5000/µL)
- At least 3 months duration
- Monoclonal
- Appropriate immunophenotype (CD19+, CD5+, CD23+, dim CD20+, dim CD22+, dim to negative CD79b, dim to negative CD81, CD43+, dim CD45+, dim surface immunoglobulin)

Diagnosis made by medical professional based upon identification of abnormal pattern and correlating this with history and other test results (WBC)
Flow Cytometry Used in Diagnosis and Sub-Classification of B- LPD

- Differentiation of reactive from clonal
- Sub-classification of CD5+ B-cell neoplasms:
  - CLL, MCL, PLL, LCL, HCL, FL,
- Sub-classification of CD10+ B-cell neoplasms:
  - FL, Burkitt’s, LCL, MCL, HCL
- Sub-classification of B-cell neoplasms with hairy/villous cytoplasmic borders
  - HCL, HCLv, SLVL
Differentiation of reactive from clonal

48% B-cells
Subclassification of B-Cell Neoplasms: CD5+

- **MCL:**
  - +CD5, +CD19, - CD23 (may be dim+)
  - moderate CD20, CD22, sIg, CD79b, CD81, CD45,

- **CLL:**
  - +CD5, +CD19, +CD23,
  - dim CD20, CD22, sIg, CD79b, CD81, CD45

- **PLL and LCL**
Subclassification of B-Cell Neoplasms: CD10 Positivity

- **FL:**
  - + CD10, dim CD19, +CD20, +CD22, +slg, +/-CD23, -CD5, -CD25, -CD11c, -CD103

- **Burkitt’s:**
  - +CD10, moderate CD19, +CD20, +CD22, +slg, -CD23, -CD5

- **ALL: Immature**
  - +CD10, dim CD19, +CD22, +/-CD20, +TdT, -slg, +/-CD34

- **LCL and MCL**
Subclassification of B-Cell Neoplasms: Hairy/Villous Cytoplasmic Borders

**HCL**
- + CD19, Bright CD20, Bright CD22, Bright CD25, Bright CD11c, CD103, CD123

**HCLv**
- + CD19, Bright CD20, Bright CD22, ++CD11c (can be moderate or dim), +CD103, - CD25, - or dim CD123

**SLVL**
- + CD19, Moderate CD20, Moderate CD22, +/- CD11c (dim when +), CD25-, CD103-, CD123-
Summary

- Flow cytometry is routinely used for diagnosis and sub-classification of B-cell neoplasia.
- The diagnosis is made by a medical professional with intimate knowledge of normal immunophenotypic patterns.
- Diagnosis is based upon recognition of an abnormal pattern, not a single value, similar to evaluation of morphology.
- Flow cytometric results are correlated with clinical history and the results of other tests for the diagnosis.