INTRODUCTION:

CD5-positive chronic lymphoproliferative disorders/lymphomas are characterized by their morphologic, immunophenotypic, and cytogenetic characteristics. In clinical flow cytometry labs, panels are designed to distinguish between the different immunophenotypic subtypes.

After identifying a population of interest, an important next step in establishing a diagnosis of a B-cell lymphoproliferative disorder is to confirm B lineage on the population. This is typically done with two B cell markers, including CD19 and CD20. One of the key reasons to include more than one B cell marker is that surface expression of either marker may change on the neoplastic populations either due to biology (i.e. low surface CD20 antigen density in some lymphoma cells) or therapy (i.e. loss of CD20 in patients treated with rituximab/anti-CD20 therapy).

Once B cell lineage is established, the next step is to query whether any of the B cells express CD5. CD5 is a T cell marker that is not typically expressed on B cells (it is only dimly expressed on a subset of late stage hematogones/normal B-lineage precursors). Selection of the appropriate antibody and fluorochrome is important, as the aberrant expression of CD5 by the clonal B cells may be dimmer than on normal T cell populations (antibody clone and fluorochrome choices are discussed in a separate module).

If there are B lineage cells that express CD5, Boolean gating can be employed to see whether the CD5-positive B cells are restricted for kappa or lambda surface light chain. The examples shown in this module have dim or normal expression of clonally restricted surface light chain; it is important to note that these cases are typical presentations. Rare cases may present that lack surface light chain (but are clonally restricted with cytoplasmic light chain analysis) or be “biclonal.” These examples are discussed in separate modules.

The following two cases provide a direct comparison of the morphology and immunophenotype of the two most common CD5-positive B cell lymphomas – chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and mantle cell lymphoma (MCL).

CASES:

1. CLL/SLL: This patient is a 60 year old woman who presented with fatigue. A CBC demonstrated leukocytosis due to an absolute lymphocytosis. On peripheral smear the cells were predominantly small, with high nuclear:cytoplasmic ratios and clumped, mature chromatin. Many smudge cells were noted. A bone marrow biopsy was performed, and there was an excess of atypical lymphocytes (Figure 1). Concurrent flow cytometry identified a CD5-positive kappa monotypic B cell population that expressed CD23, but lacked CD79b. In addition, the CD20 and surface light chain were dim positive. The morphology and immunophenotype are diagnostic of CLL/SLL. Cytogenetics/FISH can be useful in ambiguous cases, to exclude a t(11;14) and to identify genetics features more typical of CLL/SLL such as trisomy 12.
2. **MCL:** This patient is an 80 year old man that presented with weight loss and lymphadenopathy. A CBC demonstrated leukocytosis due to an absolute lymphocytosis. On peripheral smear, the cells were predominantly small to intermediate with high nuclear:cytoplasmic ratios. The chromatin was mature, the borders of the nucleus irregular. A bone marrow biopsy was performed, and there was an excess of atypical lymphocytes (Figure 2). Concurrent flow cytometry identified a CD5-positive kappa monotypic B cell population that expressed CD79b, but lacked CD23. In addition, the CD20 and surface light chain had intensity similar to normal B cells. The morphology and immunophenotype are diagnostic of mantle cell lymphoma. Typically in these cases the diagnosis is further confirmed by demonstrating a t(11;14) by FISH, conventional cytogenetics, or IHC (overexpression of the Cyclin D1 gene product).

**CD200:** CD200 can be an important marker to distinguish CLL/SLL from MCL. CLL/SLL cases typically express surface CD200, while MCL cases lack CD200.

**FMC7:** FMC7 is typically negative on CLL/SLL and positive on MCL. This antigen is an epitope on the CD20 molecule and has been described widely in the literature, it is used less frequently in clinical labs due to its perceived limited utility.

**CD22, CD81 and CD43:** These additional markers can also help distinguish CLL/SLL from MCL. CLL/SLL cases typically express CD43 and have down regulated expression of CD22 and CD81.

The key immunophenotypic differences that distinguish CLL/SLL from MCL are depicted in Figure 3. A normal profile is included in the left column. The B cells express CD20 and are polytypic for surface light chains. They lack CD5. The CD5 population depicted in the normal profile represents T cells.

**PEARLS:**

- **Chronic lymphocytic leukemia/small lymphocytic lymphoma:** CD5+, CD19+, CD20 (dim), CD23+, CD79b-/+ (partial dim), CD200+, FMC7-, monoclonal surface light chain (dim)
- **Mantle cell lymphoma:** CD5+, CD19+, CD20, CD23-, CD79b+, CD200-, FMC7+, monoclonal surface light chain
- **Monoclonal B lymphocytosis:** Typically has the immunophenotype of CLL/SLL, but the distinction between CLL/SLL and MBL is made on the basis of WBC count and clinical features (including staging). This will be discussed in a different module.
- **Equivocal/absent surface light chain:** If there is a clear population of cells that coexpress CD5 and B cell markers (including CD19 and/or CD20), some labs may repeat analyses and perform cytoplasmic light chain staining

**REPORTING:**

Examples from one lab of how CLL/SLL and MCL may be reported are provided on the next pages. Structure of reports is often restricted based on the electronic medical record (EMR) systems used (for example, some EMRs allow PDF reports with images, while others only allow HL7 text). Key elements include: 1) describing population of interest and immunophenotype, 2) enumerating population size, and 3) providing an interpretive comment. If there are multiple people in the flow cytometry lab who verify reports, uniformity among how the lab structures reports improves communication to the end user (typically a clinician reading a report within an
EMR). Reporting requirements also vary based on state and/or country specific regulations for patient safety and billing. Two key components of a report within the United States include:

1. Enumeration of all markers performed to assist with both technical and professional “per marker” billing
2. A disclaimer that analyte specific reagents (ASRs) are used for testing and generally do not require Food and Drug Administration (FDA) approval. Essentially all leukemia/lymphoma testing in the United States uses laboratory developed tests (LDTs) which are not FDA-approved but may be subject to future FDA guidance

EXAMPLE OF REPORTS

A - CLL/SLL CASE

FINAL DIAGNOSIS: Bone marrow, left, aspirate: CD5-positive kappa monotypic B cells (30%); see comment

COMMENT: The differential diagnosis includes chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), mantle cell lymphoma, and rare other entities, and the immunophenotype favors CLL/SLL.

REPORTABLE RESULTS:
- Percentages reported below are based on the total number of CD45 positive viable leukocytes. If applicable, percentage of plasma cells is from total viable nucleated cells.
- 30% B cells which express CD5, CD23, and dim monotypic kappa immunoglobulin light chains and lack CD79b. The B cells have similar forward scatter relative to background T cells suggestive of similar size; however, precise size determination is deferred to morphology.
- Polytypic B cells are rare to absent.

Additional components to be included in report (depending on location of testing):
1. Clinical history/indication for testing (helpful to assign ICD-10 code and for billing)
2. Enumeration of antibodies used for testing (billing)
3. Analyte specific reagent disclaimer (compliance)

B - MCL CASE

FINAL DIAGNOSIS: Bone marrow, right, aspirate: CD5-positive kappa monotypic B cells (35%); see comment

COMMENT: A diagnosis of mantle cell lymphoma is favored based on the bright CD20, bright immunoglobulin light chain, expression of CD79b and lack of CD23. However, correlation with additional studies (such as cytogenetics, t(11;14) FISH), and/or immunohistochemical stain for cyclin D1) are recommended to confirm this diagnosis.

REPORTABLE RESULTS:
- Percentages reported below are based on the total number of CD45 positive viable leukocytes. If applicable, percentage of plasma cells is from total viable nucleated cells.
- 35% B cells which express CD5, CD79b, and monotypic kappa immunoglobulin light chains and lack CD23. The B cells have similar forward scatter relative to background T cells suggestive of similar size; however, precise size determination is deferred to morphology.
- Polytypic B cells are rare to absent.
Additional components to be included in report (depending on location of testing):
1. Clinical history/indication for testing (helpful to assign ICD-10 code and for billing)
2. Enumeration of antibodies used for testing (billing)
3. Analyte specific reagent disclaimer (compliance)

Figure 1 – Marrow Aspirate CLL/SLL

Figure 2 – Marrow Aspirate MCL
**References:**


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