

# PEARLS OF LABORATORY MEDICINE

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**TITLE: Mature B-cell Neoplasms**

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**Slide 1:**

My name is Dr. Kamran Mirza and together with my AP/CP pathology resident Dr. Michael Moravek I would like to welcome you to this Pearl of Laboratory Medicine on “Mature B-cell Neoplasms”.

**Slide 2:**

An understanding of mature B-cell neoplasms requires a general understanding of the outline of lymphocyte development. The main cells in this system are T-cells, B-cells and NK-cells

Similar to phases of development of all hematopoietic cells, B-cell maturation starts with immature, pro-B cells and passes through several stages before culminating at the formation of the plasma cell.

This mature B-cell, highlighted in the box represents the basis of all lesions that will be discussed in this video. Immature B-cell neoplasms such as acute lymphoblastic leukemias and terminal B-cell lesions such as plasma cell neoplasms are discussed separately.

Overall, mature B-cell neoplasms account for 75% of all lymphoid neoplasms

In general, B-cell lymphomas arise as a result of genetic alterations that lead to deregulation of cell proliferation or apoptosis

Low-grade B-cell lymphomas are typically chronic diseases with a relatively indolent course. These commonly present as painless lymphadenopathy, hepatosplenomegaly or incidentally found CBC abnormalities

Higher grade, or more aggressive lesions typically present with rapidly enlarging masses or lymph nodes in addition to a spectrum of symptoms known as B-symptoms. These include fever, weight loss and night sweats

**Slide 3:**

The mature B-cell undergoes exposure to antigen in lymph nodes. This diagram gives an overview of that process.

Neoplastic change can occur at any point along this path and the subsequent clonal proliferation demonstrates similar morphology and phenotype as it's cell of origin

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## Mature B-cell Neoplasms

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Generally, pre-germinal center B-cells are naïve cells that have yet to encounter antigen. Such naïve cells are the cell of origin of mantle cell lymphoma

Similarly, there is a variety of germinal center derived lymphomas that can be identified on morphologic and phenotypic findings including Burkitt lymphoma, follicular lymphoma and some diffuse large B-cell lymphomas.

Marginal zone B-cell derived lymphomas arise from marginal zone cells that are usually seen in the spleen and pelvic lymph nodes

Post-germinal B-cell neoplasms include the activated B-cell or ABC type diffuse large B-cell lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), marginal zone lymphoma of mucosa-associated lymphoid tissue, or MALT lymphoma and plasma cell neoplasms.

Various stages of B-cell development can be identified by morphology as well as their changing antigen expression phenotype.

Immunohistochemical analysis helps us subclassify various mature B-cell lymphomas and allows differentiation from immature B-cell lesions.

### **Slide 4:**

Over the past few decades, our understanding of the biology, pathophysiology, and prognosis of B-cell neoplasms has greatly improved. Putting this understanding to good use, there have been several classification schemes for lymphomas. Older classification systems defined lesions based on morphology alone.

Over time, classification schemes evolved to incorporate clinical features, immunophenotype, cytogenetic, and molecular genetic data. Here is the most current list of mature B-cell neoplasms as defined by the most recent edition of the WHO classification of Tumors of hematopoietic and lymphoid tissue published in 2017.

In this video, we will be reviewing the lesions highlighted here. Our discussion will commence with the most frequently encountered lesions.

Reported data shows diffuse Large B-cell lymphoma is the most common, accounting for just under 30% of all B-cell neoplasms

CLL/SLL, follicular lymphoma, marginal zone lymphoma, mantle cell lymphoma and Burkitt lymphoma follow DLBCL in decreasing frequency

The first step in determining the presence of a B-cell neoplasm is identifying the presence of a clonal population of B cells. This can be done by observing light chain restriction via flow cytometry or immunoglobulin heavy chain rearrangement studies. When a clonal population of B-cells is identified, subclassification is based on the lymphoma cell morphology and their immunophenotype.

This algorithm represents an easy way to approach B-cell neoplasm by their antigen expression

The CD5 positive B-cell lymphomas include CLL/SLL and mantle cell lymphoma – These two entities are most often differentiated based on the expression of Cyclin D1 and CD23 or FMC7 and the intensity of CD20 expression.

The follicular center cell origin, or CD10 positive lesions can be differentiated on size, morphology and architectural patterns.

We start our discussion with CLL/SLL. A CD5 positive, CD23 positive mature B-cell neoplasm

### **Slide 5:**

Chronic lymphocytic leukemia and Small lymphocytic lymphoma, CLL/SLL define the same lymphoma, but are named based on whether the lymphoma is mostly in leukemic phase, which is CLL, or present as a nodal or tissue lesion, where it is called small lymphocytic lymphoma. The WHO classification collectively refers to these lesions by CLL/SLL

CLL/SLL accounts for the most frequently encountered lymphoma in western countries

This is usually a lymphoma of older patients, with a median age of 70, who usually present with unintentional weight loss, fatigue, and fever. Sometimes, CLL/SLL is discovered in patients with no definitive symptoms

To make a diagnosis of CLL, the CBC and differential should reveal an absolute lymphocytosis with greater than 5000 lymphoma cells per microliter

CLL/SLL is not defined by specific genetic alterations; however arises as a multistep process of genetic changes, and commonly demonstrates cytogenetic abnormalities

### **Slide 6:**

SLL can have a vaguely nodular appearance. At low power pale zones can be appreciated, as seen in the image on the left. These are pseudo proliferation growth centers. This pallor is due to a greater proportion of larger prolymphocytes with abundant cytoplasm in these regions.

In the peripheral blood, CLL cells have high N:C ratio, irregular condensed chromatin that is often referred to as having a soccer ball pattern. Blood smears from CLL patients often demonstrate numerous smudge cells, or basket cells.

Phenotypically, CLL/SLL is a CD5 positive B-cell lymphoma. It usually demonstrates dim expression of CD19, CD20 and CD5

CD23 positivity and Cyclin D1 negativity differentiate CLL/SLL from Mantle cell lymphoma

Large/confluent proliferation centers and proliferation centers with high mitotic activity are adverse prognostic indicators.

### **Slide 7:**

A series of adverse prognostic factors have been associated with CLL/SLL.

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These include high stage disease, high levels of beta 2 microglobulin, Unmutated immunoglobulin heavy chain and expression of CD38, ZAP70 and CD49d

Cytogenetic or FISH assessment of deletions of 11q and 17p and molecular assessment for mutations of TP53, BIRC, Notch1 and SF3B1 is important

Large cell transformation of CLL/SLL is known as Richter's transformation. Most factors that predispose to Richter's transformation are similar to the adverse prognostic factors of CLL/SLL

### **Slide8:**

Apart from CLL/SLL the differential diagnosis of CD5 positive B-cell lymphoma includes the CD23 negative, Cyclin D1 and FMC7 positive mantle cell lymphoma

Mantle cell lymphoma is also a disease of older adults. Patients typically present with adenopathy, hepatosplenomegaly and marrow involvement. This is a disease with poor prognosis.

Two MCL subtypes recognized with different clinicopathological manifestations and molecular pathogenetic pathways: one largely with unmutated/minimally mutated IGH and mostly SOX11+ and the other largely with mutated IGHV and mostly SOX11- (indolent leukemic non-nodal MCL with PB, bone marrow (BM), ±splenic involvement, may become more aggressive)

The t(11;14)(q13;q32) translocation between the IGH and CCND1 genes is found in >95% of cases and leads to overexpression of the cyclin D1 protein. This portends to a better prognosis.

### **Slide 9:**

Arising from the mantle zones around the germinal center, mantle cell lymphoma is comprised of mature, slightly irregular lymphocytes.

Sox11 is the most sensitive monoclonal antibody and is seen in >90% of mantle cell lymphoma, including the rare CD5 negative mantle cell lymphomas

### **Slide 10:**

We now turn our attention to the CD5 negative, CD10 positive follicular lymphoma.

FL is a germinal center origin lymphoma that is usually seen in older patients who can be asymptomatic despite widespread disease. This is generally a chronic, low grade process. However, progression to GCB type DLBCL is one of the known adverse outcomes. While usually a nodal disease, extranodal FL can involve the spleen, marrow, blood, tonsils and other tissues

The underlying genetic alteration in FL is translocation of the IgH and BCL-2 genes on chromosomes 14 and 18 leading to an inappropriately high rate of bcl-2 transcription for a mature B cell. BCL-2 overexpression leads to diminished apoptosis of abnormal B-cells within germinal centers that lead to the proliferation of a malignant clone.

### **Slide 11:**

As the name suggests, Follicular lymphoma demonstrates a proliferation of back-to-back follicles devoid of tingible body macrophages.

Follicular centers are composed of a mixture of centrocytes and centroblasts. Follicular lymphoma is graded by counting the number of centroblasts per high power field in 10 neoplastic follicles. If this number is greater than 15, it is considered a grade 3, or high grade FL.

The CD20 positive B-cells of follicular lymphoma are CD10 and BCL-6 positive cells that express BCL-2, consistent with neoplastic follicular cells.

This video has described garden variety follicular lymphoma. It should be noted that specific subtypes such as Pediatric-type follicular lymphoma, duodenal-type FL, and in situ follicular neoplasia exist; however, are outside the scope of today's discussion.

### **Slide 12:**

As their name suggests, diffuse large B-cell lymphomas are composed of diffuse sheets of large, clonal B-cells. Immunophenotypically, DLBCL can come in two main groups, the CD10 positive and CD10 negative. These phenotypic differences have prognostic relevance.

As previously discussed, nodal and extranodal DLBCLs account for the most number of B-cell neoplasms

This diagnosis is usually made in adults, with a mean age of 70 years

In general, these lesions present abruptly, are high-grade and carry a poor prognosis

From a biologic perspective, DLBCLs can arise de novo from their cell of origin, represent a large-cell transformation from a low-grade B-cell lymphoma, or arise in the setting of immune suppression or chronic inflammation

Distinction of germinal center b-cell type versus activated B cell type, which is also referred to as non-GCB type, DLBCL is important for prognostication

Rearrangement or immunophenotypic expression of MYC and BCL2 are also important determinants of clinical outcome

### **Slide 13:**

Gene expression profiling studies have identified two broad categories of DLBCL, germinal center B-cell or GCB type and Non-GCB, or activated B-cell, ABC type.

The GCB and ABC categories can be delineated based on this immunohistochemical algorithm proposed by Hans and colleagues in 2004.

The decision tree is initiated with evaluating the CD10 stain.

Positivity for CD10 in DLBCL defines the germinal center B-cell type of DLBCL

CD10 negativity leads to evaluation of BCL-6.

Broadly speaking, CD10 and BCL-6 negative DLBCLs are non-GCB or ABC type

In the case that a CD10 negative DLBCL is BCL-6 positive, the decision for GCB vs. ABC is dependent on MUM1 expression

In the R-CHOP era, both the 5-year progression-free survival and overall survival rates demonstrate a statistically significant difference between GCB and non-GCB/ABC with GCB type reflecting better outcomes in both survival plots.

### **Slide 14:**

Diffuse large B-cell lymphoma reveals sheets of large cells. Nodal architecture is effaced by a diffuse proliferation of these cells. This is contrasted with the normal, follicular architecture shown above.

Large cell lymphoma cells are usually 2 or more times larger than small reactive lymphocytes. Here this difference is easily appreciated between the large cells on the right of the image and the small lymphocytes streaming in the middle/left.

A small battery of immunostains is typically used to sub-characterize these lymphomas and to relay prognostic information and in some cases therapeutic options to the clinician.

In the interest of time and focus, our discussion today centered around GCB and non-GCB/ABC type large B-cell lymphomas; however, it should be mentioned that the large B-cell lymphoma family is a heterogeneous group of entities. Other large B-cell lymphomas include T-cell/histiocyte-rich large B-cell lymphoma, primary DLBCL of the CNS, primary cutaneous DLBCL leg type, primary mediastinal large B-cell lymphoma of putative thymic origin, intravascular B-cell lymphoma and ALK positive diffuse large B-cell lymphoma to name a few.

### **Slide 15:**

Our third CD10 positive lymphoma is Burkitt lymphoma

Burkitt lymphoma is a highly aggressive but curable lymphoma with a long-term overall survival rate of 70-90%. Three variants of BL have been identified: Endemic, Sporadic and Immunodeficiency-associated. Given its propensity to double in size very quickly, this lesion is often an emergency if growing near vital structures.

The endemic variant is primarily seen in equatorial Africa and New Guinea, has a peak incidence of 4-7 years of age. The sporadic variant is primarily seen in the USA and Western Europe and has a peak incidence of 11 years of age.

The immunodeficiency-associated variant is more common in the setting of HIV infection than in other forms of immunosuppression. In HIV-infected patients, Burkitt lymphoma appears early in the progression of disease when CD4 T-cell counts are still high.

### Slide 16:

Burkitt lymphoma is comprised of intermediate sized cells with squared off borders. The mitotic rate and Ki-67 index is extremely high. Scattered, pale tingible macrophages amongst the lymphoma cells gives rise to the famous starry sky appearance of this lymphoma.

While there is no one feature that defines BL, appropriate morphology, high proliferative index and rearrangement of the MYC gene on chromosome 8 are features often looked at when making this diagnosis.... MYC rearrangement is commonly t(8;14) that involves the MYC gene and immunoglobulin heavy chain, and less frequently t(2;8) or t(8;22) in which MYC rearrangement is with either immunoglobulin kappa and lambda light chains respectively

A subset of lymphomas with resemble Burkitt lymphoma in morphology, phenotype and microRNA and gene expression profiling but LACKING the MYC rearrangement has been described as Burkitt-like lymphoma with 11q aberration. The clinical course of this entity seems to be similar to that of Burkitt lymphoma.

### Slide 17:

Marginal zone lymphoma is a CD5 and CD10 negative mature B-cell neoplasm

Like the other low-grade lymphomas discussed before, Marginal zone lymphoma is a disease of older adults and can be subclassified as mucosa associated lymphoid tissue lymphoma (also known as MALT lymphoma), nodal marginal zone or splenic marginal zone lymphoma based on the localization of the lymphoma cells.

Several translocations, some of which are listed here, may be seen, but none are pathognomonic.

Of note, translocation 11, 18 leads to API2 - MLT gene fusion. This translocation is associated with low-grade MALT lymphoma of the stomach and of the lung. Importantly, this translocation is associated with resistance to H Pylori eradication therapy.

### Slide 18:

Marginal zone lymphoma typically demonstrates small, mature B-cells with rare interspersed larger cells. A common feature of MALT lymphomas that is highlighted here is the presence of lymphoepithelial lesions where lymphocyte infiltration is noted to be destroying epithelial cells.

In general, marginal zone lymphoma is negative for CD5, CD10 and CD23.

### Slide 19: References

This marks the end of this pearl of laboratory medicine on B-cell neoplasms. In this video we discussed the main diagnostic algorithms and basic descriptions of the more common B-cell neoplasms. References for this presentation are listed here.

### Slide 20: Disclosures

The authors have no disclosures or potential conflicts of interest.

Slide 21: Thank You from [www.TraineeCouncil.org](http://www.TraineeCouncil.org)

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Thank you for joining me on this Pearl of Laboratory Medicine on “Mature B-cell Neoplasms”.

