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Immunohistochemistry

Immunohistochemistry in the Evaluation of Follicular or Nodular Lymphoid Lesions

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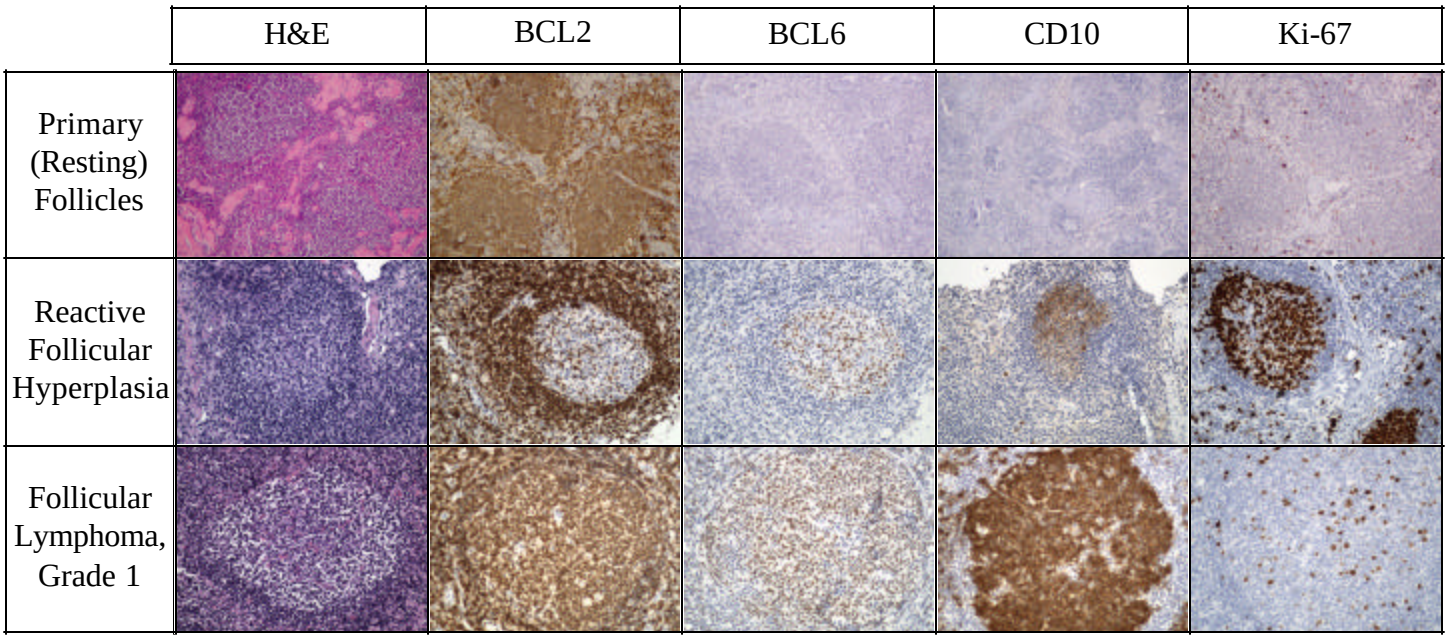
This month, we briefly review the use of immunostains in evaluating several lymphoid lesions with a nodular or follicular growth pattern. Immunostains are often essential for determination of their nature and clinical significance.

Before discussing the utility of particular immunostains, it is important to review and understand some basics about the normal lymphoid follicle, in order to interpret the immunostains appropriately. An important point to understand is the difference between a primary follicle and a secondary follicle. A primary or "resting" follicle is a collection of B-cells in the cortex of the lymph node that has not been antigenically stimulated, and because of this, it does not have a germinal center. Once a primary follicle has been antigenically simulated, it acquires a germinal center, and at this point is referred to as a secondary follicle.

When faced with a lymphoid lesion with follicular structures or nodules, the main possibilities to consider in the differential diagnosis include resting (primary) follicles, secondary follicles (as a reflection of reactive follicular hyperplasia), follicular lymphoma, and mantle cell lymphoma with a nodular growth pattern. On a few occasions, B-cell small lymphocytic lymphoma/CLL with prominent pseudofollicular proliferation centers may also enter into the differential diagnosis. Many times the distinction of non-neoplastic lymphoid follicles vs. neoplastic lymphoid follicles can be readily made on standard H&E morphology alone. As we all know however, we see cases where this distinction is challenging, and in other situations we are faced with minuscule or suboptimal material in which artifacts of suboptimal fixation or tissue processing interfere with our ability to appreciate satisfactory morphologic detail. By understanding the expected phenotype of the



lymphoid cells within each of the various types of lymphoid follicles, one can frequently render a confident diagnosis, even in the face of suboptimal material or a minimal biopsy sample.

The lymphocytes in a primary (resting) follicle express B-cell markers (such as CD20, CD79a, or Pax-5) and BCL2. CD23 may be positive or negative, but they lack BCL6, CD10, CD5, and cyclin D1, and they have a low Ki-67 proliferative fraction. In contrast, in the germinal center of a secondary follicle, the lymphoid cells lack BCL2, CD5, CD23, and cyclin D1, express both BCL6 and CD10, and have a very high proliferative fraction, approaching 100%. In follicular lymphoma, the neoplastic cells express B-cell markers, BCL2 and BCL6, and lack CD5, CD23 and cyclin D1, with a variable Ki-67 proliferative fraction. Most but not all follicular lymphomas express CD10, and some grade 3 follicular lymphomas lack BCL2. In grade 1 follicular lymphoma, a low proliferative fraction is observed, in grade 2 follicular lymphoma a moderate proliferative fraction, and in grade 3 follicular lymphoma a high proliferative fraction. In mantle cell lymphoma, the lymphocytes express B-cell markers, BCL2, CD5, and cyclin D1, typically lack BCL6 and CD23, and generally show a moderate Ki-67 proliferative fraction. And finally, in B-cell small lymphocytic lymphoma/CLL, the neoplastic cells express B-cell markers, CD5, CD23, and BCL2, but lack BCL6, cyclin D1, and CD10. The proliferative fraction is generally low to moderate, depending on the number of associated large cells within proliferation centers.

Since some of these follicular or nodular structures to some extent consist of mixed B and T-cell populations, there are often minor populations of cells staining for the markers above that I listed as lacking, and for that reason in some cases it is easiest to interpret the immunostain results on low-power. For example, in the case of primary (resting) follicles, there are often a small number of background cells that may express BCL6, CD10, and CD5. It is always a good idea to compare the extent of reactivity of the markers discussed with the extent of reactivity of the associated B-cell markers. Finally, it must be kept in mind that CD23 stains a subset of the follicular dendritic cells (FDC) that may be present in these conditions, so care must be taken to not misinterpret CD23 reactivity of FDC as reactivity of the lymphocytes. Finally, it is worthwhile to note that some follicular lymphomas contain impressive numbers of non-neoplastic T-cells.

Another point that must be made is this: "tumors do not read textbooks". As such, not all lymphoid proliferations will neatly fit into the expected patterns of reactivity discussed above. For example, we have seen clear-cut cases of both follicular lymphoma and mantle cell lymphoma that have expressed strong CD23, a few mantle cell lymphomas that have expressed BCL6, and we have even seen a rare case of follicular lymphoma that expressed CD5.

	Primary (Resting) Follicle	Germinal Center (Reactive Follic. Hyp.)	Follicular Lymphoma, Grade 1	Follicular Lymphoma, Grade 2	Follicular Lymphoma, Grade 3	Mantle Cell Lymphoma	B-cell SLL / CLL
CD20, CD79a	POS	POS	POS	POS	POS	POS	POS
BCL2	POS	neg	POS	POS (few -)	POS or neg	POS	POS
BCL6	neg	POS	POS	POS	POS	neg	neg
CD10	neg	POS	POS	POS (few -)	POS or neg	neg	neg
Ki-67	Low	Very High	Low	Moderate	High	Mod - High	Low - Mod
CD23	POS or neg	neg (FDC+)	neg (FDC+)	neg (FDC+)	neg (FDC+)	neg (FDC+)	POS (few-)
CD5	neg	neg	neg	neg	neg	POS	POS
Cyclin D1	neg	neg	neg	neg	neg	POS	neg

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