

## **Diagnosing Gastric MALT Lymphoma: Update and Handling of Follow up Biopsies**

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### **Background**

It has long been known that the stomach is the most common location of lymphoma within the gastrointestinal tract. However, it is only in the past few decades that the reason for this fact has been found. The discovery of gastric *Helicobacter pylori* infection and its role in the pathogenesis of chronic gastritis, lymphoma and probably gastric carcinoma has revolutionized our definition of malignancy. The concept of a pathway of lymphoid hyperplasia (chronic gastritis) leading to lymphoid “dysplasia” (atypical lymphoid infiltrates) that further evolves into a proliferation that fulfills criteria for malignancy (lymphoma) has been compared to the adenoma-carcinoma sequence in epithelial neoplasia. What is unique to gastric MALT (or marginal zone) lymphomas is the ability to halt and reverse the process by eradication of *H. pylori* with antibiotics in most cases.

This lecture and handout will review the pathogenesis and histologic diagnosis of gastric MALT lymphoma arising in the setting of *H. pylori* associated gastritis. The use of immunohistochemical stains and PCR to detect clonal lymphoid populations in the initial diagnosis will be discussed. A special emphasis will be placed on how to handle follow up biopsies from patients previously treated for low grade gastric MALT lymphoma, either by antibiotics or chemotherapy.

### **Pathogenesis of Gastric MALT Lymphoma**

A number of studies have established the likely pathogenesis of MALT lymphoma in the setting of *H. pylori* gastritis. Infection results in the recruitment of inflammatory cells to the gastric mucosa (acquired mucosa associated lymphoid tissue or MALT). T lymphocytes within the infiltrate mount an antigen specific immune response against the organism and secondarily induce a B-lymphocyte antibody response. In most cases the B cell response is polyclonal and is dependant on continued T-cell stimulation. In some patients, chronic ongoing stimulation over many years results in the development

of a monoclonal B-cell population. Even at this stage, simple antibiotic therapy to eradicate *H. pylori* (hence removing the source of antigenic stimulation) is curative in 60-90% of cases. Persistence of lymphoma or progression from low grade to high-grade morphology is associated with the acquisition of additional molecular abnormalities, such as t (11:18), TP53 inactivation, p16 deletion and c-myc translocation. However, these tests are not usually needed in the diagnosis of high-grade lymphoma.

### **Histologic Diagnosis**

The histologic diagnosis of gastric MALT lymphoma is usually made on endoscopic gastric mucosal biopsies. The endoscopic appearance of MALT lymphoma varies, and includes the finding of near normal mucosa, severe “gastritis”, thickened folds and large mass lesions. Large malignant appearing ulcers due to lymphoma are less commonly found than in the past. This may be due to the modern practice of eradication of *H. pylori* in many people at the stage of chronic gastritis, and to earlier detection and treatment of low grade MALT lymphoma in western countries where there is easy access to endoscopy.

The classic morphology of low-grade gastric MALT lymphoma includes:

- Dense lamina propria infiltrates composed of small atypical lymphoid cells
  - These are usually found in a background of chronic gastritis with numerous plasma cells
  - The atypical infiltrates typically expand and efface the mucosal architecture, with sheets of monomorphic cells infiltrating between gastric glands.
  - Benign lymphoid follicles with germinal centers are frequent, or may contain atypical lymphoid cells (“colonization” by lymphoma)
  - There may be a mixture of small and large atypical cells, or predominantly large cells (high grade MALT lymphoma)
  - The presence of *H. pylori* in the majority of patients
- Lymphoepithelial lesions
  - Consist of lymphocytes infiltrating gastric glands and destroying the mucosal architecture

- Can be highlighted with keratin stains if needed to visualize partially destroyed gastric glands
- When numerous biopsies are taken, the changes are frequently patchy among fragments, with some biopsies showing only chronic gastritis with *H. pylori*.

When the classic morphology is present the diagnosis can be confidently made on H and E stains alone. However, in most practices it is considered the standard of care to perform immunohistochemical stains in order to phenotype lymphocytes. In some centers, PCR analysis to detect monoclonal heavy chain B-cell gene rearrangements is also routine. However, if the H and E and immunohistochemical staining pattern are characteristic of MALT lymphoma, molecular analysis is not usually necessary.

### **Immunohistochemical Staining Pattern**

CD20 and CD3 stains readily highlight the proportion of B and T cells present and serve as a quick confirmation that low grade MALT lymphoma is present in histologically classic cases. The finding that the atypical lymphoid component is composed of CD20 positive B-cells with scattered mature T-cells admixed is typical. However, if there is a desire to exclude mantle cell or follicular lymphoma, additional immunostains are needed. The following constellation of markers is useful in paraffin sections:

- Low grade MALT lymphoma (most common in stomach)
  - Positive for: CD20 and CD43 +/-, BCL2 immunostaining can also be positive (not BCL-2 gene rearrangements)
  - Negative for: CD5, CD10, BCL-1
- Mantle cell lymphoma (uncommon in stomach, but can occur)
  - Positive for: CD19, CD20, BCL-1 (cyclin D1)
  - Also co-express the T-cell markers CD5 and CD43
  - Negative for: CD10, CD23 and BCL-2
- Follicular lymphoma (very uncommon in stomach)
  - Positive for: CD10, CD20, BCL-2 and BCL-6 (not usually used)
  - Negative for: CD5, BCL-1

## **Molecular Tests**

As in all ancillary testing, the decision to request a molecular test requires an understanding of how the results will assist in the diagnosis. Molecular test results must always be interpreted in the context of all other available histological, immunohistochemical and clinical information.

PCR analyses for B-cell heavy chain gene rearrangements are positive in low-grade gastric MALT lymphoma (and in most other B-cell lymphomas). However, depending on the primers used, there is a 30% false negative rate for the test. Therefore, a positive result confirms a diagnosis of lymphoma in most cases, while a negative result does not absolutely exclude lymphoma. PCR analysis is most helpful when the diagnosis is uncertain between severe chronic gastritis and lymphoma.

BCL-1 and BCL-2 gene rearrangement tests are negative in MALT lymphoma. These tests are usually performed to evaluate for either Mantle cell (BCL-1) or follicular (BCL-2) lymphoma.

### **Differential Diagnosis: Gastritis or Low Grade MALT Lymphoma?**

Distinguishing severe chronic gastritis from low grade MALT lymphoma is the most common diagnostic dilemma in gastric biopsies containing dense lymphocytic infiltrates. In practice, the distinction between the two can often be easily accomplished histologically. In more difficult cases, immunohistochemistry and PCR analysis may be required to arrive at the correct diagnosis. In the following discussion, bare in mind that lymphoma occurs in a background of chronic H. pylori related gastritis. Therefore, a mixture of gastritis and lymphoma in biopsies is the rule, not the exception.

Chronic gastritis related to H. pylori is characterized histologically by the presence of a mixed inflammatory infiltrate with a predominance of plasma cells, **not** lymphocytes, as in MALT lymphoma. Eosinophils and neutrophils are commonly present, frequently infiltrating the gastric glands. While gland destruction is often present, complete effacement of the glandular architecture is less commonly seen in active gastritis. Lymphoid aggregates with germinal centers may be present.

Immunohistochemical stains in the setting of gastritis reveal a mixture of T- and B-lymphocytes, with appropriate zonation noted in areas of lymphoid aggregates. Diffuse sheets of B-lymphocytes are not generally found. The numerous plasma cells

present in gastritis do not stain with either CD20 or CD3. Therefore, much of the infiltrate is negative if stains are limited to these two markers. CD20 positivity that is limited to lymphoid aggregates lessens the likelihood that lymphoma is present. Kappa and lambda stains can be applied and will show a mixed population of plasma cells in gastritis, but these stains are not usually needed. In cases where the histology and immunohistochemistry fail to yield a definite diagnosis, molecular analysis for clonality can be used as the final arbiter. Again, the interpretation of PCR analysis should always take into account the complete clinical and histologic picture. When in doubt, additional biopsies can be recommended following treatment for H. pylori.

### **Proper Handling of Follow Up Biopsies After Therapy**

Following a diagnosis of low grade MALT lymphoma, the first line of therapy in patients with H. pylori infection consists of eradication of the organism with a combination of antibiotics and proton pump inhibitors. In patients who are H. pylori negative by all techniques, standard chemotherapy will be utilized as initial therapy. In either case, it is important that clinicians not re-biopsy too soon after therapy, in order to avoid over-diagnosing regressing disease as residual disease requiring additional therapy. In general, follow up biopsies should be taken no sooner than 3-6 months after therapy is initiated.

The histology of post treatment biopsies falls into three categories:

- Definitely negative for residual lymphoma
  - Biopsies should show little to no lymphoid infiltration, with no atypical lymphoid cells seen. Lymphoid aggregates composed of small, mature lymphocytes may be present. The presence of other inflammatory cells (plasma cells, neutrophils and eosinophils) is of no clinical relevance.
  - Immunohistochemical stains and molecular studies are not recommended in histologically negative biopsies.
  - If the endoscopic appearance or any other clinical test is suspicious for residual lymphoma, additional biopsies and endoscopic ultrasound are needed to exclude sampling error.

- Recommendation: Continued routine follow up at an appropriate interval (e.g., one year).
- Definitely positive for residual lymphoma
  - Biopsies show evidence of lymphoma readily on H and E sections.
  - Immunohistochemistry can be used as needed to confirm the diagnosis.
  - PCR analysis is not necessary, but can be useful to compare clones between original and follow up biopsies.
  - At this point, chemotherapy or radiation therapy is generally recommended.
- Indefinite for residual lymphoma
  - Biopsies show focal lymphoid infiltrates that are suspicious but are insufficient to render a diagnosis of residual lymphoma on histologic grounds.
  - This is a common scenario that requires the use of immunohistochemistry and PCR analysis to clarify.
  - Important Note: In patients treated with anti-CD20 antibodies (Rituxan), CD20 stains will be negative in residual tumor due to antibody blocking CD20 sites. Therefore, negative CD20 staining does not exclude lymphoma. Other B-cell markers (PAX 5) may be used.
  - If PCR confirms the presence of a clonal B-cell population, continued therapy depends on the overall clinical picture. If there is no endoscopic evidence of disease, clinical follow up without additional therapy may be recommended.

**Significance of Residual B-cell Clones in “Negative” Biopsies (Residual Molecular Disease)**

- Recently, persistence of monoclonal B cell populations in patients without clinical or histologic evidence of lymphoma after H. pylori eradication has been reported.

- While the clinical significance of these persistent clones is still unclear, in one follow-up study, recurrent disease was significantly more likely to develop in patients with persistent clones as compared to those who were polyclonal by PCR. However, only 3 of 14 patients ultimately relapsed.
- In a separate follow up study, 5 of 7 patients with persistent B-cell clones had negative or indefinite histology immediately after therapy. 3 of 7 patients ultimately relapsed.
- Relapses/recurrences were local, not systemic in these patients, although progression to high grade lymphoma occurred in some.
- Continued, long term follow up of patients with residual molecular disease is recommended. However, there appears to be no indication for chemotherapy in patients whose only evidence for disease is molecular.

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