“Adieu to disappointment and spleen…”

For many centuries the spleen was considered to be the organ of bad temper, and the word “spleen”, as noted in the above quote from Jane Austen’s *Pride and Prejudice*, was once used as a synonym for spite or anger.¹ Splenic pathology challenges even the most experienced pathologist, sometimes conjuring up the very feelings cited in the title above. The pathologist’s task is particularly daunting in the setting of inadequate clinical history, poor specimen handling (e.g. poorly fixed tissue), or lack of suitable tissue for ancillary studies, such as flow cytometry or cytogenetics. This article will highlight a few of the more common and recently described lymphoproliferative disorders involving the spleen and review optimal specimen handling to help assure appropriate interpretation and subsequent patient care.

The most frequently encountered indications for splenectomy include the following: incidental (such as part of a distal pancreatectomy or gastrectomy specimen), trauma, myeloproliferative neoplasms, lymphoproliferative disorders, inflammation/infection, metabolic diseases, hypersplenism-related disorders (such as idiopathic thrombocytopenia purpura, congestive splenomegaly due to portal hypertension, and hereditary spherocytosis), and diagnosis of a “splenic mass” discovered by CT or MRI.²

A retrospective review of splenectomy specimens received in the Rex Pathology Laboratory over a 12 year period (January 1, 2000 to January 1, 2013) yielded a total of 285 cases. The breakdown for the diagnostic categories of these cases is shown in the following table and chart.

### Rex Pathology Splenectomy Diagnoses 2000-2012

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal/Incidental</td>
<td>91</td>
</tr>
<tr>
<td>Hypersplenism</td>
<td>67</td>
</tr>
<tr>
<td>Congestive splenomegaly</td>
<td>44</td>
</tr>
<tr>
<td>ITP</td>
<td>22</td>
</tr>
<tr>
<td>Hereditary Spherocytosis</td>
<td>1</td>
</tr>
<tr>
<td>Hematologic neoplasm</td>
<td>53</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>29</td>
</tr>
<tr>
<td>Myeloproliferative neoplasm</td>
<td>23</td>
</tr>
<tr>
<td>Granulocytic sarcoma</td>
<td>1</td>
</tr>
<tr>
<td>Trauma</td>
<td>37</td>
</tr>
<tr>
<td>Infection</td>
<td>13</td>
</tr>
<tr>
<td>Vascular tumor</td>
<td>9</td>
</tr>
<tr>
<td>Metastatic malignancy</td>
<td>7</td>
</tr>
<tr>
<td>Cyst/Pseudocyst</td>
<td>5</td>
</tr>
<tr>
<td>Metabolic disease</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total cases</strong></td>
<td><strong>285</strong></td>
</tr>
</tbody>
</table>

Thus, approximately 20% of the splenectomy cases were hematologic neoplasms, and of these, 55% were lymphoproliferative disorders. The lymphoproliferative disorders consisted predominantly of marginal zone lymphoma (nine cases), large B-cell lymphoma (six cases), and follicular lymphoma (three cases). These three lymphomas also comprised
the most common lymphomas documented in another larger case series of patients (without a previous diagnosis of lymphoma) undergoing diagnostic splenectomy for unexplained splenic mass or splenomegaly.

One of the diagnostic dilemmas pathologists face in evaluating splenectomy specimens is differentiating follicular or marginal zone lymphoma from reactive follicular or marginal zone hyperplasia. Morphologic clues, such as a lack of tingible body macrophages in the germinal centers of follicles, can point towards a follicular lymphoma rather than hyperplasia. **However, immunophenotypic analysis, preferably by flow cytometry, is often essential in establishing the diagnosis.** Immunohistochemical evaluation of paraffin sections can be helpful in selected cases of lymphoproliferative disorders. Aberrant expression of bcl2 in CD10 or bcl6 positive germinal centers, is suggestive of follicular lymphoma. However, a study of splenic follicular lymphoma in the *American Journal of Surgical Pathology* showed only 12 cases (of the 32 studied) were bcl2 positive, while 20 of the follicular lymphomas were bcl2 negative or showed only weak/partial bcl2 expression. The bcl2 positive cases tended to be low grade and CD10 positive, whereas the bcl2 negative cases were high grade and CD10 negative/positive. The bcl2 negative cases were also more commonly primary splenic follicular lymphomas. In marginal zone hyperplasia versus marginal zone lymphoma, where there is expansion of the marginal zone in both entities, some morphologic features may point towards one entity over the other, such as the lack of coalescence of the adjacent white pulp segments in marginal zone hyperplasia. **But here again, flow cytometry is often essential to confirm the presence of a monoclonal B cell population.** Some cases may require molecular evaluation for B-cell gene rearrangement to accomplish this. Furthermore, flow cytometry studies not only serve to differentiate benign hyperplasias from lymphoproliferative disorders by establishing clonality, they also assist in appropriate classification. Both follicular and splenic marginal zone lymphomas can display an expanded marginal zone, but by immunophenotypic analysis, follicular lymphomas usually show a CD5- CD10+ monoclonal B cell population, whereas, marginal zone lymphomas often demonstrate a CD5-CD10- monoclonal B-cell population.

This patient underwent a therapeutic splenectomy for idiopathic thrombocytopenic purpura (ITP)

Another area that can be challenging in splenic hematopathology involves the diffuse red pulp small B-cell lymphocytic infiltrates, which include hairy cell leukemia, splenic marginal zone lymphoma, and the provisional entities, hairy cell leukemia variant and splenic diffuse red pulp small B-cell lymphoma. The latter two entities fall under the designation “Splenic B-cell lymphoma/leukemia, unclassifiable” in the current WHO classification of hematopoietic neoplasms. The morphology of these entities can be similar, but again the flow cytometric findings help to differentiate these processes. For example, hairy cell leukemia is CD25+, whereas the other lymphoproliferative disorders are negative for this antigen. Cytochemical staining for tartrate-resistant acid phosphatase (TRAP) can also be helpful in establishing the diagnosis of hairy cell leukemia. Differentiating hairy cell leukemia from the other entities is important since it shows unique sensitivity to treatment with cladribine.

Splenic diffuse red pulp small B-cell lymphoma is rare (<1% of non Hodgkin lymphomas). It is a leukemic neoplasm, involving the spleen, bone marrow, and peripheral blood. In fact, the peripheral blood often shows villous lymphocytes similar to those reported in splenic marginal zone lymphoma. Involvement of the spleen is characterized by a diffuse expansion of the red pulp, including cords and sinusoids, by a monotonous population of small round lymphocytes with pale cytoplasm. The disease is typically indolent and no treatment may be necessary, though a subset of patients may benefit from splenectomy. Flow cytometric studies showing an absence of CD103 expression may help to distinguish this entity from hairy cell leukemia variant, but overlap of these two entities has

**Extensive red pulp replacement by a proliferation of histiocytes, suggestive of a metabolic disorder, such as Gaucher disease.**

**Extramedullary hematopoiesis in a myeloproliferative neoplasm**
been described. Hairy cell leukemia variant is also uncommon. Similar to splenic diffuse red pulp small B-cell lymphoma, it affects the spleen, bone marrow and peripheral blood. In the spleen, there is diffuse red pulp expansion by a lymphocytic infiltrate morphologically similar to typical hairy cell leukemia, including small cells with a moderate amount of pale cytoplasm, imparting a “fried egg” appearance. By immunophenotyping, CD25 is negative and the cells are TRAP negative. This process is also typically indolent with monoclonal antibody therapies directed against CD20 and CD22 showing efficacy. Splenectomy has been cited as an alternative treatment.4

Marginal zone lymphoma (left) versus marked marginal zone hyperplasia (right). In both processes, there is expansion of the marginal zone; therefore, immunophenotyping by flow cytometry is often essential to differentiate these entities.

Diffuse large B-cell lymphoma: H&E sections (left) showed effacement of the normal splenic architecture by a population of large atypical lymphocytes which were diffusely CD20 positive by immunohistochemistry (right)

*Immunophenotyping by flow cytometry cannot be performed on formalin-fixed tissue; therefore, it is important to always send splenic specimens FRESH to the surgical pathology laboratory.* If there is a clinical suspicion of a lymphoproliferative process, please request an “Intraoperative Pathology Consult for Lymphoma Work-up” on the requisition. The pathologist will perform touch imprints and place fresh tissue in RPMI media for flow cytometry and cytogenetic studies as needed. Subsequent overnight formalin fixation of thinly sliced sections allows for optimal histologic evaluation and additional immunohistochemical studies.

Peripheral blood and bone marrow involvement by a patient with Splenic B-cell lymphoma/leukemia, unclassifiable. The peripheral blood showed villous lymphocytes and the bone marrow exhibited a sinusoidal and interstitial infiltrate of small atypical lymphocytes with moderate pale cytoplasm. The infiltrate was diffusely CD20 positive and further immunophenotyping studies by flow cytometry were performed to aid in the diagnosis

Questions regarding optimum specimen handling are welcome and should be directed to the pathologist covering “OR Consults/Frozen Sections” for the originating Operating Room.

Preeti H. Parekh, M.D.

Specimen Triage for Lymphoma Work-up
Carbapenem-resistant Enterobacteriaceae (CRE) Screening at Rex

Starting in July 2013 Rex will begin screening patients for Carbapenem-resistant Enterobacteriaceae (CRE).

Background
The CDC definition of CRE is Enterobacteriaceae that are nonsusceptible to one of the following carbapenems: doripenem, meropenem, or imipenem AND resistant to all of the following third-generation cephalosporins that were tested: ceftriaxone, cefotaxime, and ceftazidime. Klebsiella species and Escherichia coli are the most common CRE, but Enterobacter, Serratia, and Citrobacter strains have also been reported.

CRE may have a high mortality rate (up to 50%). CRE frequently carry resistance genes to other antimicrobials making them difficult to impossible to treat.

The most common mechanism of resistance in CRE is production of a Klebsiella pneumonia carbapenemase (KPC). KPC was first detected in the United States in 2001. Worldwide there are other mechanisms of carbapenem resistance including New Delhi metallo-B-lactamase (NDM), Verona integrin-encoded metallo-B-lactamase (VIM), and imipenemase metallo-B-lactamase (IMP). These are found predominantly outside the US and are usually associated with inpatient care.

Screening at Rex
All patients entering Rex Healthcare will be asked: Have you been hospitalized outside the United States within the last six months? All “yes” respondents will have a screening culture and be placed on contact isolation precautions. The screen for CRE is a rectal swab. (The Rex Hospital Infection Control Service will be responsible for collecting the specimen.) Once a result comes back as negative for CRE the patient can be removed from isolation.

Laboratory Procedure for detection of CRE
Once the lab has received a rectal swab with an order for CRE screen the swab will be plated on a chromID CARBA agar which is a selective chromogenic medium for the screening of carbapenemase-producing Enterobacteriaceae. The culture plates will be read after 18-24 hours and negative results reported. If there is growth suggestive of CRE additional workup will be done including overnight incubation followed by an oxidase test. Positive results will be reported as CRE screen positive. These positive screen cultures will be forwarded to Mayo Clinic for confirmation of carbapenemase production and identification of resistance mechanism. The CRE screening program will be overseen by Rex Infection Control.

Summary
The initial patient screen will be the question, “Have you been hospitalized outside the United States within the last six months?” Only those who answer “yes” (a very small fraction of Rex patients) will have a rectal swab to see if the patient is colonized with CRE. Those that are colonized with CRE will remain on contact isolation during their stay at Rex.

Vincent Smith, M.D.
Susan Tricas, M.T.

References
1. Chromogenic medium for the isolation of carbapenem-resistant Enterobacteriaceae; bioMérieux IFU 16900B; 2012/05.
3. Laboratory protocol for detection of carbapenem-resistant or carbapenemase-producing, Klebsiella spp. and E. Coli from rectal swabs; www.cdc.gov/HAI/pdfs/labsettings/Klebsiella_or_Ecoli.pdf