SPLEEN: CASE STUDIES

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The aim on this lecture is to present a pragmatic diagnostic guide to tumorous lesions in the spleen, whether reactive or neoplastic and not to provide an update or a compendium of the disorders that commonly involve the splenic white and red pulp. The lecture is based on four case studies that are employed to illustrate a practical approach to solving the issue of newly discovered splenic masses and to advance a comprehensive review of the unique splenic manifestations of the specific disease entities that comprise the four cases.

CASE #1

Clinical History

Splenomegaly without lymphadenopathy was discovered in a 57-year-old female. A bone marrow study disclosed atypical lymphocytic nodules that were both intertrabecular and paratrabecular. The patient subsequently underwent splenectomy; the spleen weighed 1,098 grams.

Discussion

Microscopic sections reveal obvious involvement of the spleen by malignant lymphoma with variable tumorous-type expansion of the splenic white pulp. Some white pulp segments contain a monomorphous population of lymphocytes with ovoid to reniform nuclei and abundant pale-staining, almost lucent cytoplasm; these cells resemble monocytoid cells and are found not only in the white pulp, but also diffusely infiltrating the red pulp as well as forming small tumor nodules in the red pulp. A minority of white pulp segments have "biphasic" appearance with the monocytoid-like cells found at the periphery or marginal zone areas of the expanded white pulp, whereas the central portions contain darker-staining lymphocytes with crowded nuclei and less visible cytoplasm. The latter areas resemble primary follicles or mantle zones. There are no apparent residual germinal centers. In addition, the largest tumorous-appearing nodules in the white pulp contain large lymphocytes which form clusters and aggregates. In contrast to the neoplastic monocytoid-appearing cells in the marginal zones, the large cells have vesicular nuclei with prominent nucleoli and are associated with increased mitotic activity. The morphologic features conform to splenic marginal zone lymphoma (SMZL) with focal transformation to large cell lymphoma.

Immunophenotypic analysis employing flow cytometry disclosed a monoclonal B cell population with kappa light chain restriction. The neoplastic lymphocytes did not exhibit coexpression for CD5 and CD3 reactivity also was negative, as was CD10. Immunoperoxidase studies verify that the SMZL, including the large cell component, is of B-cell lineage with strong reactivity for CD20, but not for cyclin D1 (*bcl*-1). Enlarged lymph nodes dissected from the

splenic hilum also were involved by lymphoma consistent with marginal zone lymphoma; the lymph nodes did not exhibit transformation to large cell lymphoma.

SMZL is an indolent lymphoma that reposits in the splenic white pulp to form microscopic nodules, similar to those in other lymphomas. The lymphoma usually has either a marginal zone or a biphasic pattern. The marginal zone pattern is characterized by a proliferation of neoplastic marginal zone lymphocytes, that frequently are monocytoid-like, together with occasional blast-type cells and plasma cells which broaden the marginal or parafollicular zones of the white pulp, encircling and infiltrating residual atrophic-appearing germinal centers. The neoplastic marginal zone cells, unlike those in mantle cell lymphoma (MCL), often are separated from the germinal centers by residual dark-staining mantle cell lymphocytes; the well defined lucent cytoplasm of monocytoid-like lymphocytes also contrasts with the darker appearing and more crowded nuclei of the mantle cells. As in this case, with more extensive involvement, SMZL expands beyond its apparent *in situ* position in the splenic marginal zones to form a series of coalescent tumor nodules which completely replace the white pulp and secondarily invade the red pulp; the red pulp infiltrate resembles hairy cell leukemia (HCL). In some cases, SMZL may have a distinct lymphoplasmacytic component; such cases often are associated with autoimmune disorders, especially autoimmune hemolytic anemia, and monoclonal gammopathy, as in Waldenström macroglobulinemia (WM). WM and SMZL with plasmacytic features share morphologic and many immunophenotypic characteristics and are distinguishable only by clinical and detailed molecular cytogenetic analyses. Transformation of SMZL to large cell lymphoma also may occur. A peripheral lymph node is most common site of transformation, but as illustrated in case 1, large cell lymphoma also can originate in the spleen, in association with splenic MZL.

The biphasic pattern is characterized by an enlarged central core of small lymphocytes, similar to the cells of mantle cell lymphoma, encircled by a peripheral ring of marginal zone-type cells. Both the central and peripheral regions are regarded as part of the same neoplastic clone. The majority of MZL cases in the spleen have a biphasic pattern. The inclusion of the biphasic cases in the category of SMZL arose out of a review of the splenic histologic findings of 37 cases documented as **splenic lymphoma with villous lymphocytes** (SLVL). SLVL is a chronic lymphoproliferative disorder predominantly occurring in elderly men with consistent massive splenomegaly, minimal lymphadenopathy, and circulating atypical lymphocytes with cytoplasmic villous projections that often are localized to one pole of the cell. A monoclonal spike is found in the serum or urine in about two-thirds of patients. Unlike HCL, the lymphocytes of SLVL are tartrate-resistant acid phosphatase negative and cases usually do not express CD25, CD103, CD123, HC2 or annexin A1. About 15% of patients have a translocation of t(11;14)(q13;q32) and rearrangement of the *bcl*-1 locus with increased expression of cyclin D1; the latter patients may have a more aggressive clinical course and represent leukemic MCL cases that simulate SMZL/SLVL. Currently, the patients with clinical SLVL are considered to have a leukemic form SMZL.

A predominant red pulp or diffuse pattern of infiltration has been described in cases thought to represent SMZL, but the 2008 WHO classification places such cases in the provisional category of "splenic diffuse red pulp small B-cell lymphoma."

SMZL is a B-cell neoplasm expressing various B-cell antigens, IgM and IgD monoclonal immunoglobulin, and *bcl*-2 protein; CD5, CD10, CD23, CD43, and cyclin D1 generally are not reactive. The absence of CD5 and CD43 expression, as well as cyclin D1, eliminates chronic

lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and MCL from the diagnosis, and lack of CD10 excludes follicular lymphoma (FL). Despite the designation "marginal zone" for this distinct splenic lymphoma, SMZL differs genetically from its extranodal namesake. Similar to node-based MZL, but unlike extranodal MZL of MALT, the splenic cases do not have detectable t(11;18) translocations or *API2-MALT1* fusion transcripts; however, some patients with splenic MZL display genetic losses and such losses, specifically 7q deletion, are associated with shorter survival. In this regard, the cases with a biphasic pattern do not appear to derive from the marginal zone, but may arise from a novel type of B cell located in the mantle zone. In fact, molecular analysis suggests that there are two types of SMZL, one that originates from naïve marginal zone B cells and one that derives from memory marginal zone B cells, with antigen selection as a possible factor in lymphomagenesis. In this regard the stimulating antigen in some examples of SLVL may be hepatitis C (HCV); approximately 20% of patients with SMZL have associated HCV and mixed cryoglobulinemia and evidence a hematologic response to antiviral theapy to imply that HCV has a significant role in lymphomagenesis. The unmutated cases of SLVL are associated with the 7q deletion and resultant shorter progression-free interval and overall survival in comparison to patients with mutated IgV_H genes. Microarray analysis of SMZL demonstrates a homogeneous signature that differs from other small B-cell lymphomas, specifically by the expression of the *ILF1*, *SENATAXIN* and *CD40* genes.

Although uncommon, p53 abnormalities can occur in SMZL and also are associated with aggressive disease. In general, however, SMZL is an indolent lymphoma as patients have a better prognosis than nodal MZL patients with a median time to progression of 6.9 years in contrast to 1.3 years for those with node-based MZL. In one recent report from Italy of 309 patients with SMZL, 72% of the patients were alive, five years following diagnosis and 65% remained alive at 10 years. For patients with SMZL who progress to large B-cell lymphoma, the survival at years is reported as 55%.

Four months following splenectomy, this patient developed generalized lymphadenopathy. A lymph node biopsy obtained from the left side of the neck demonstrated diffuse large cell lymphoma; a low-grade marginal zone component was not present.

Diagnosis

Splenic marginal zone lymphoma with focal transformation to large B-cell lymphoma.

CASE #2

Clinical History

The patient, a 34-year-old female, presented with a three month history of fever, weight loss, sweating, and vomiting associated with splenomegaly, but without lymphadenopathy. Bone marrow examination and flow cytometry studies were negative for any definite malignancy. A 1,136 gram spleen was resected.

Discussion

The splenic architecture is altered by a series of irregularly spaced nodules involving both white and red pulp together with diffuse infiltration of the splenic cords and sinuses. The

cell population is extremely heterogeneous. The majority of lymphocytes are small to medium-sized including some with irregular nuclear membranes. There also are many pleomorphic large cells. The latter generally have prominent nucleoli and some have lobated nuclear contours. Some lymphomatous-type nodules are altered by hemorrhage and degeneration and many associated histiocytes are found. The presence of multiple heterogeneous splenic nodules is characteristic of malignant lymphoma. Although Hodgkin lymphoma (HL) should be considered, the lack of Reed-Sternberg cells militates against that diagnosis. The pattern of a series of irregular nodules involving both white and red pulp with involvement of the periarteriolar lymphoid sheaths, together with areas of hemorrhage, necrosis, and a histiocytic reaction including erythrophagocytosis usually is found in peripheral T cell lymphomas (PTCL). However, morphologically identical cases represent examples of large cell lymphomas of T cell/histiocyte-rich B-cell type. In this case, flow cytometry analysis disclosed a monoclonal kappa light chain population and by immunoperoxidase, the large cells proved to be of B-cell lineage with strong reactivity for antigen CD20. In contrast, the large cells did not express CD79a, whereas the smaller lymphocytes are T cells showing variable staining for antigens CD3, CD43, and CD45RO. The admixed histiocytes exhibited the expected reactivity for CD68 and no positive reaction for *bcl*-2 protein was discovered.

T cell/histiocyte-rich B-cell lymphomas (T/HRBCL) presenting in the spleen are unusual, but these lymphomas are similar to those developing in lymph nodes and are as equally heterogeneous. In addition to granulomatous inflammation, HL and PTCL, the differential diagnosis of T/HRBCL also includes T-cell lymphoma with epithelioid histiocytes (Lennert lymphoma). In classic Lennert lymphoma, the neoplastic lymphocytes often are found in the T zones of the spleen, namely the periarteriolar lymphoid sheaths and/or marginal zones. In the latter site, epithelioid histiocytes frequently form a ring-like array around the white pulp. Some cases of T/HRBCL in the spleen contain markedly anaplastic large cells raising the possibility of anaplastic large cell lymphoma (ALCL), Primary splenic ALCL is rare; one case was described in a patient with HIV infection and other cases are described with spontaneous splenic rupture and with symptoms of a splenic abscess.

A recent study of 17 cases of T/HRBCL presenting in the spleen indicates that these cases display a micronodular pattern involving the white pulp and red pulp. The cases may be associated with fibrosis, necrosis and hemorrhage, with some large cells resembling Reed-Sternberg cells. Immunophenotypic studies on paraffin sections are mandatory for an accurate diagnosis; the micronodules comprise numerous CD3-reactive T cells, CD68-positive histiocytes, and randomly dispersed large cells expressing CD20 with immunoglobulin light chain restriction. The prognosis in these cases is poor.

Large B-cell lymphoma (LBCL) is the most common primary lymphoma in the spleen. Similar to SMZL, hepatitis C virus may have a role in the lymphomagenesis of splenic LBCL. Classically, it appears as a large tumor mass or a series of large, confluent nodules extensively replacing the splenic parenchyma and secondarily invading the red pulp. The lymphoma frequently breaches the capsule to invade contiguous organs. The distribution appears completely random, and the neoplastic cells are often adjacent to uninvolved segments of white pulp.

Primary splenic LBCL cases include not only those with large coalescent tumor masses but also those with multiple, discrete monomorphous nodules in the centers of the white pulp, an association with CLL/SLL, FL of grade 1 or 2 type, or SMZL, and a centroblastic or

immunoblastic plasmacytoid cytology. Some LBCL cases may manifest initially in the marginal zone, resulting in the formation of crescent-shaped infiltrates around intact follicles. Rare cases of LBCL may also primarily reposit in the splenic red pulp with diffuse infiltration of the cords and, occasionally, the sinuses. In addition to B-cell antigens, the diffuse red pulp splenic LBCL cases may coexpress CD5. If the large B cells are predominately distributed in the red pulp sinuses, such cases may represent a version of intravascular large B-cell lymphoma, and, principally in Asia, may be affiliated with a hemophagocytic syndrome and aggressive clinical behavior. Currently, the diagnosis of splenic involvement by intravascular LBCL requires confirmation by the detection of extrasplenic intravascular lymphoma.

Diagnosis

T cell/histiocyte-rich large B cell lymphoma.

Case #3

Clinical History

Following a morphologically nondiagnostic bone marrow study, a 3,223 gram spleen was removed from this 57-year-old male.

Discussion

Histologic sections of this enormous spleen disclose a diffuse red pulp infiltrate by small, lymphocytes. No visible white pulp is apparent. The white pulp is virtually replaced and obliterated by the diffuse red pulp lymphocytic infiltrate, which fills both cords and sinuses. The lymphocytes are small to medium-sized cells with coarse chromatin, including some with stippled chromatin. The cytoplasm is variable, but in areas, the cytoplasm is abundant and pale-staining to impart a monocytoid appearance. Occasional lymphocytes have slightly irregular nuclear contours and some appear plasmacytoid. Scattered larger cells with small nucleoli are evident ("blasts"). Mitotic activity is uncommon. Small red cell "lakes" and "pseudosinuses" that are partially lined by the infiltrating lymphocytes are observed similar to those associated with HCL.

Immunophenotypic analysis by flow cytometry disclosed a monoclonal B cell population with lambda light chain restriction. The neoplastic B cells did not express CD5, CD10 or CD23, but reacted for CD19, CD20, FMC-7 and CD11c. Unlike the usual form of HCL, no reactivity for CD25 and CD103 was found. Immunoperoxidase stains on the paraffin sections revealed that, as in the flow cytometry analysis, the neoplastic lymphocytes expressed B-cell antigen CD20 as well as CD79a. IgD also was expressed. In contrast to HCL, the lymphocytes were DBA.44 negative and B-cell antigen CD45RA (4KB5) proved only to be sporadically and weakly positive; however, the infiltrating lymphocytes showed intense positivity for tartrate-resistant acid phosphatase (TRAP). A small minority of the lesional cells expressed bcl-2 and stains for both bcl-6 and cyclin D1 were negative. Proliferative activity as analyzed by Ki-67 expression was low and noted in 5-10% of lymphocytes.

Cytogenetic studies of the spleen revealed a normal male karyotype and FISH analysis was negative for rearrangement of the MALT1 gene.

Histologically, this case is indistinguishable from HCL, the prototype lymphocytic malignancy that preferentially diffusely infiltrates the red pulp cords and sinuses. In HCL, the white pulp may be completely obliterated or it may appear atrophic and encroached upon by the infiltrating hairy cells. The subendothelium of the trabecular veins is consistently infiltrated. Hairy cells in the spleen are cytologically bland and homogeneous. Mitotic figures are uncommon. The nuclei usually are oval or reniform, and the cytoplasm is characteristically abundant and lucent, often with well-delineated cell borders. The presence of variably dilated sinuses filled with red cells ("lakes") and lined by hairy cells ("pseudosinuses") is another characteristic morphologic feature of spleens involved by HCL. In rare cases, the blood lakes may be so prominent as to resemble a cavernous hemangioma. Red cell lakes, however, are not pathognomonic of HCL, and, on occasion, may be found in other leukemias involving the spleen, such as CLL/SLL. In CLL/SLL, however, careful morphologic examination generally reveals an expanded white pulp that often is masked by the diffuse red pulp infiltrate. Red cell lakes also occur in so-called hairy cell leukemia variant (HCL-V) and the newly proposed splenic diffuse red pulp small B-cell lymphoma. No absolute morphologic features are established to allow the distinction of HCL from HCL-V in the spleen and from splenic diffuse red pulp small B-cell lymphoma.

In sections, hairy cells express B-cell antigens, including CD20 and CD79a, but not CD5, CD10, or CD23. They also react with monoclonal antibody DBA.44, express cyclin D1 and are tartrate-resistant acid phosphatase (TRAP) positive. CD123, annexin A1, and the T-cell associated transcription factor T-bet are newer markers that expedite the diagnosis of HCL in paraffin sections with annexin A1 being virtually specific for HCL. Employing flow cytometry, the hairy cells strongly express CD11c, CD25 and CD103.

The immunophenotype of HCL-V equally mimics HCL with the expression of CD11c and CD103, but, unlike typical HCL, the variant cells are CD25 negative and do not express CD123 or annexin A1. In point of fact, the most challenging differential diagnosis in the spleen rests not between HCL and HCL-V or HCL and splenic diffuse red pulp small B-cell lymphoma, but between HCL-V and the splenic diffuse red pulp small B-cell lymphoma; moreover, it remains contentious whether HCL-V and splenic diffuse red pulp small B-cell lymphoma are actually discrete malignancies. In both neoplasms, the histologic features in the spleen and immunophenotype are interchangeable, although the pattern of bone marrow involvement appears different, as does the coexpression of preswitched with postswitched immunoglobulin heavy chain isotypes found in HCL-V.

Despite of the overall morphologic similarity of the spleen in case #3, to HCL and the reactivity for TRAP, the immunophenotypic profile, specifically the absence of reactivity for CD25 and CD103, is unlike HCL. TRAP positivity, moreover, is not specific and may be observed in B-cell lymphoproliferative disorders apart for HCL. The TRAP positivity in this case and lack of CD103 expression also differs from the variant form of HCL-V. The immunophenotypic profile in case #3 is identical to the nonspecific immunophenotype found in SMZL and cases such as this one could conform to the putative rare diffuse variant of SMZL. The four diffuse variant cases of SMZL reported, however, were IgD negative in contrast to the current case.

In the 2008 WHO classification scheme, cases such as case #3 are best regarded as "splenic B-cell lymphoma/leukemia, unclassifiable" and likely represent a permutation of

"splenic diffuse red pulp small B-cell lymphoma," a provisional category of indolent lymphoma. This form of lymphoma encompasses the cases that were formerly designated as the "diffuse red pulp variant of SMZL" and more recently reported as "splenic red pulp lymphoma with numerous basophilic villous lymphocytes." These cases are associated with peripheral blood involvement, often with villous type-lymphocytes, as well as bone marrow involvement. In the spleen, the red pulp cords and sinuses are diffusely infiltrated by small to medium-sized, generally round lymphocytes admixed with occasional large cells. As discussed above, similar to HCL and HCL-V, pseudosinuses and red cell lakes may be present and the white pulp usually is atrophic or obliterated. The neoplastic lymphocytes express CD20 and DBA.44, but unlike HCL, they are CD11c, CD25, CD103, CD123 and annexin A1 negative. Similar to SMZL, splenic diffuse red pulp small B-cell lymphoma does not express CD5, CD10 or CD23. The cells are IgG positive and may also express IgD. TRAP staining is not found. Cases often exhibit IgH mutations. Complex cytogenetic alterations have been described, but they do not exhibit del 7q and t(11;14). The current case differs from the descriptions of splenic diffuse red pulp small B-cell lymphoma in the WHO classification in that DBA.44 reactivity was negative while the TRAP stain was positive. Nonetheless, splenic diffuse red pulp small B-cell lymphoma is a provisional category, and although CD103 negative, displays considerable overlap with HCL-V so that additional cases and investigation are required to further delineate these uncommon lymphomas/leukemias.

Diagnosis

Splenic B-cell lymphoma/leukemia, unclassifiable (? splenic diffuse red pulp small B-cell lymphoma).

Case # 4

Clinical History

A splenic nodule was discovered by ultrasound in a 27-year-old male who was investigated for epigastric pain. At surgery, a well-circumscribed 6 cm. in diameter tumor mass was found in the spleen which weighed 385 grams. The mass had a variegated pink-tan surface with bright yellow necrotic areas.

Discussion

In contrast to the adjacent unremarkable splenic white and red pulp, the mass is relatively solid and lacks white pulp. It is composed of a mixture of small lymphocytes and plasma cells including cells with Russell bodies. There also are admixed immunoblasts and larger mononuclear cells in keeping with histiocytes. None of the histiocytes appear "foamy" or lipid-laden and no evident granulomas are identified. Depending on the area, irregular small bands of fibrous connective tissue are noted and fibrous connective tissue also partially demarcates the mass from the adjacent spleen.

Immunoperoxidase studies disclosed patchy collections of CD20 reactive B cells. The B cells include the small to medium-sized lymphocytes, as well as some immunoblasts. The plasma cells are polyclonal expressing both kappa and lambda light chains. Most small lymphocytes are of T-cell lineage reacting for CD3 and CD8, whereas the histiocytes express CD68 including

cells with dendritic-like processes. Unlike the usual form of hamartoma, no reactivity for CD8 was discovered, except among lymphocytes and reactivity for Factor VIII is limited to scattered endothelial cells. Flow cytometry studies also demonstrated a polyclonal B lymphocytic population with unremarkable T cells. Based on both morphology and immunohistochemistry, the mass fits best with an inflammatory pseudotumor.

Inflammatory pseudotumors of the spleen are unusual lesions that involve the splenic red pulp and form prominent masses replacing the parenchyma to simulate malignant lymphoma Similar to this case, the pseudotumors are composed of a heterogeneous proliferation of various reactive cells with a typical zonal distribution. Although not prominent in this case, pseudotumors typically have variably dense spindle shaped fibrous connective tissue and central necrosis with hemorrhage and cholesterol cleft formation may be observed. These areas are surrounded by lymphocytes, sheets of plasma cells, fibroblasts, and even epithelioid granulomas. The morphologic features are similar to those of inflammatory pseudotumors in other locations and must be distinguished from a malignant process including malignant lymphoma, mainly HL. Unlike lymphoma, the small lymphocytes are predominately reactive CD8 positive T cells and the plasma cells are polyclonal. B cells are infrequent in contrast to histiocytes. The spindle cells are mostly vimentin-positive fibroblasts, but some spindle cells are myofibroblasts that express smooth muscle actin, whereas others are CD68-positive spindled histiocytes. The spindle cells also may be EBV positive, and in one case, the EBV genome was proven to be clonal. The pathologic features are similar to those of inflammatory myofibroblastic tumors in soft tissue, except that the lesions in the spleen do not react for anaplastic lymphoma kinase. Inflammatory pseudotumors must be distinguished from a malignant process, such as a follicular dendritic cell sarcoma or tumor with a prominent chronic inflammatory cell reaction Patients with inflammatory pseudotumor who undergo splenectomy show no evidence of recurrence or subsequent development of a hematopoietic neoplasm.

Inflammatory pseudotumors are one of a variety of splenic, generally non-hematopoietic tumors ranging from splenic cysts, hamartomas, vascular neoplasms (hemangiomas, littoral cell angiomas, and angiosarcomas), nonvascular mesenchymal tumors, and even metastases. Sclerosing angiomatoid nodular transformation (SANT) is a recently described non-neoplastic vascular lesion of the spleen that has been proposed to represent an unusual transformation of the red pulp in response to an exaggerated stromal proliferation. SANT comprises a series of angiomatoid nodules embedded in a dense fibrosclerotic stroma. The angiomatoid nodules are composed of slitlike, variably irregular vascular spaces that are lined by ovoid, benign-appearing endothelial cells; the vascular lumens are either empty or contain erythrocytes. The histologic and immunophenotypic features of SANT differ from other angiomatous splenic lesions, such as hemangiomas, including littoral cell angiomas, and from inflammatory pseudotumors. They also differ from a splenic hamartoma, although the likelihood that SANT is a sclerotic variety of hamartoma, or a sclerotic version of an organized hematoma or a even sclerotic inflammatory pseudotumor, cannot be entirely excluded. Similar to the current case, most cases of SANT as well as most other solitary nonhematopoietic tumors are discovered as an incidental mass.

Diagnosis

Inflammatory pseudotumor.