Splenic marginal zone lymphoma: a hydra with many heads?

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(Related Original Article on page 604)

In this issue of the Journal, Baseggio *et al.*[1] report on a series of 24 patients with CD5-positive, t(11;14)-negative splenic marginal zone lymphoma (SMZL) diagnosed by means of cytology and flow cytometry of peripheral blood. All the patients were splenectomized at diagnosis or during follow-up and, consequently, spleen specimens were available for histological examination in all cases. The biological features of the CD5-positive SMZL cases did not appear to be different from those of a comparative series of 42 CD5-negative SMZL cases with the exception of a tendency to a more mutated immunoglobulin variable heavy chain genes (IGHV) status. Clinically, CD5-positive SMZL were characterized only by more marked lymphocytosis at diagnosis and more frequent diffuse bone marrow infiltration. No significant differences were found in outcome.

Marginal zone lymphomas

The marginal zone is an anatomically distinct B-cell compartment that surrounds the lymphocytic corona of the mantle. In the past, cases of lymphoma considered to be derived from monocytoid/marginal zone B cells have been described,[2] including cases primarily involving extranodal sites. The 1992 updated Kiel classification[3] first

included the monocytoid B-cell lymphoma (and the lymphoma of mucosa-associated lymphoid tissue – MALT) among low-grade neoplasms.

The latest World Health Organization (WHO) classification[4] defines three subtypes of marginal zone B-cell lymphomas: *splenic B-cell marginal zone lymphoma, nodal marginal zone lymphoma*, including a pediatric form, and *extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue*. In addition, two provisional entities are listed: *splenic diffuse red pulp small B-cell lymphoma and hairy cell leukemia variant*. The exact relationship of these provisional entities to each other and to SMZL remains to be determined.

Splenic marginal zone lymphoma

Splenic marginal zone lymphoma is a rare, indolent subtype of lymphoma which accounts for less than 2% of all non-Hodgkin's lymphomas.[5] The presence of circulating villous lymphocytes defines splenic lymphoma with villous lymphocytes.[6] Symptomatic splenomegaly is the presenting feature in almost all patients; anemia-related symptoms and B symptoms are rare. The disease commonly pursues an indolent course with the median overall survival exceeding 10 years, but the disease can follow a more aggressive course in a significant subset of patients. The *Integruppo Italiano Linfomi* identified a prognostic score for SMZL.[7] Based on three variables (hemoglobin level less than 12 g/dL, lactate dehydrogenase level higher than normal, and albumin concentration less than 3.5 g/dL), patients were grouped into three prognostic categories: a low-risk group (41%) with no adverse factors, an intermediate-risk group (34%) with one adverse factor, and a high-risk group (25%) with two or three adverse factors. The 5-year lymphoma-specific survival rate was 88% for the low-risk group, 73% for the intermediate-risk group, and 50% for the high-risk group. An international, collaborative effort to improve the clinical usefulness and reproducibility of prognostic assessment in SMZL is much needed.

Another important issue is the role of hepatitis C virus (HCV) infection in SMZL. In our national survey in Italy, the HCV serological status was known for 255 SMZL patients (83%) and was positive in 49 of these (19%).[7] Intriguingly, French authors reported on a series of 18 patients with splenic lymphoma with villous lymphocytes associated with type II cryoglobulinemia and HCV infection,[8] some of whom were responsive to antiviral treatment.

Spleen histology. A central zone of small lymphoma ("lymphocyte-like") cells surrounds or replaces reactive germinal centers in the white pulp of the spleen. This zone merges with a peripheral area of medium-sized (monocytoid/marginal zone-like) cells and scattered larger blasts (Figure 1). Subsequently, the lymphoma progresses to the red pulp in the form of a patchy or micronodular infiltrate, which often spreads to the sinuses. Scattered histiocytes may be observed in the lymphoid aggregates. In some cases lymphoma nodules only consist of marginal zone-like cells (Figure 2).[9,10] Lymphoma cells may show a variable degree of plasmacytic differentiation, which is often prominent in cases associated with autoimmune disorders (e.g., autoimmune hemolytic anemia).[11] Splenic hilar lymph nodes may be involved, in which case they contain a vaguely nodular infiltrate based on pre-existing follicles. Lymphoma cells are represented by small lymphocytes, similar to those seen in the center of splenic nodules, which surround and/or replace germinal centers. Plasmacytic differentiation must be distinguished from lymphoplasmacytic lymphoma, whereas in the case of a follicular pattern, a follicular lymphoma should be excluded (follicular colonization *versus* neoplastic follicles).[12] Transformation to large B-cell lymphoma may occur as for other indolent B-cell lymphomas.[13]

Bone marrow histology. Bone marrow involvement by SMZL is usually in the form of a micronodular, interstitial and, sometimes, paratrabecular infiltrate. A peculiar intrasinusoidal pattern of involvement has also been observed.[14] In our study on bone marrow histology in a series of 120 marginal zone lymphomas[15] we observed an intrasinusoidal pattern in 74% of SMZL but also in 57% of nodal marginal zone lymphomas. Exclusively sinusoidal bone marrow localization is infrequent and the detection of a high rate of sinusoidal infiltration in more than one half of nodal marginal zone lymphomas is also a notable finding because the nosological definition of SMZL with adenopathy and its biological and clinical relationship with 'true' nodal marginal zone lymphomas are still matter of debate.

Flow cytometry. SMZL express surface IgM and IgD and are $CD19^+$, $CD20^+$, $CD22^+$, $CD79a^+$, $FMC7^+$, $CD10^-$, $CD123^-$, and $CD103^-$; DBA44, CD11c, CD23 and CD5 can be positive in a subset of cases. Cyclin D1/BCL-1 is not expressed, whereas the BCL-2 protein is intensely expressed.[16] According to the scoring system for chronic lymphocytic leukemia, the scores for most, if not all, cases of SMZL range from 0 to 2, a phenotype not seen in chronic lymphocytic leukemia.[17]

Immunoglobulin genes. Studies of immunoglobulin heavy-chain variable genes (IGHV) in SMZL have provided evidence that this entity is heterogeneous with respect to the preferential use of the IGHV repertoire, as well as the mutation load. In a series of 59 patients IGHV1, IGHV3, and IGHV4 gene families accounted for 30%, 56%, and 14% of sequences, respectively. The most frequently used IGHV genes were IGHV1-2 (n=12), IGHV3-23 (n=15), IGHV3-30 (n=7) and IGHV4-34 (n=5). IGHV was not mutated in 25% of the cases.[18]

Cytogenetics. The most frequent cytogenetic aberrations in SMZL are deletion of 7q and gain of 3q (nearly one third of cases). Other cytogenetic alterations are abnormalities +3, +5, +9q, +12q, +18, and +20q.[16]

We performed a genome-wide array comparative genomic hybridization at high resolution in 34 patients with SMZL. The most frequent copy number alterations involved chromosomes 7 and 17 (21% and 24%, respectively). Unmutated status of the IGVH gene was related to del(7q) and dup(12q). The high-risk group identified according to the prognostic score for SMZL was associated with del(7q) and del(17p).[19]

Diagnostic assessment of SMZL. Spleen histology is the gold-standard approach to establish a clear-cut diagnosis of SMZL. However, not all patients need splenectomy as a therapeutic approach and some are not suitable for surgery; in these cases, a combination of bone marrow histo-

logical findings and the typical clinical presentation and phenotype can be useful to corroborate the clinical diagnosis of SMZL, thus avoiding splenectomy for diagnostic purposes.

According to the proposed criteria of the SMZL working party,[16] minimum diagnostic criteria for SMZL are either: (i) spleen histology plus immunophenotype with a "chronic lymphocytic leukemia" score of two or less, or (ii) typical blood and bone marrow morphology plus immunophenotype plus intrasinusoidal infiltration by CD20-positive cells (if spleen histology is unavailable). It should, however, be underlined that the presence of intrasinusoidal bone marrow infiltration is highly suggestive of, but not diagnostic for SMZL, because a similar pattern of bone marrow involvement has also been observed in other low-grade B-cell lymphomas.[20]

Provisional entities

The 2008 WHO classification of lymphomas includes two provisional entities (*splenic diffuse red pulp small B-cell lymphoma and hairy cell leukemia variant*) of small B-cell lymphomas involving the spleen which do not fall into any of the other types of B-cell lymphoid neoplasms recognized in the classification. Other splenic small B-cell lymphomas not fulfilling the criteria for either of these two provisional entities or for other better established B-cell lymphomas should be diagnosed as *splenic B-cell lymphoma/leukemia, unclassifiable*.

Splenic diffuse red pulp small B-cell lymphoma

Splenic diffuse red pulp small B-cell lymphoma is an uncommon subtype of lymphoma in which the splenic red pulp is diffusely involved by a neoplastic population of monomorphous small B-lymphocytes.[21,22] Traverse-Glehen *et al.* recently described a series of 37 cases that might be ascribed to this entity.[21] The patients were predominantly elderly men and presented with moderate lymphocytosis and splenomegaly without pancytopenia. Spleen sections were available for only 12 patients and histology showed atrophic white pulp and a monomorphic diffuse lymphoma infiltration in a congested red pulp. Bone marrow infiltration was interstitial and intrasinusoidal. Peripheral blood was characterized by the presence of basophilic villous lymphocytes. On the basis of their findings, the authors proposed considering these cases as a provisional entity that might be called *splenic red pulp lymphoma with numerous basophilic villous lymphocytes*.

Hairy cell leukemia variant

Hairy cell leukemia variant is a rare B-cell disorder which accounts for 10% of cases of hairy cell leukemia. It affects elderly or middle-aged males. The main features are splenomegaly, lymphocytosis and cytopenias without monocytopenia. The circulating cells show morphological features that are intermediate between those of prolymphocytes and hairy cells. The immunophenotype is that of mature B cells, with expression of the B-cell antigens CD11c and CD103 but, unlike typical hairy cell leukemia cells, the cells of hairy cell leukemia variant do not express CD25. Splenic histology shows a pattern of red pulp involvement similar to that of hairy cell leukemia and splenic diffuse red pulp lymphoma, whereas recent data suggest a distinct predilection for nearly exclusively sinusoidal infiltration in bone marrow biopsies. Nearly a fifth of hairy cell leukemia variant cases showed no evidence of somatic hypermutation in their IGHV.[23] The median survival of patients with this lymphoma is 9 years and 42% of patients die of causes unrelated to the malignancy.[24]

Splenic lymphoma/leukemia, unclassifiable

According to the 2008 edition of the WHO classification, splenic small B-cell lymphomas not fulfilling the cri-

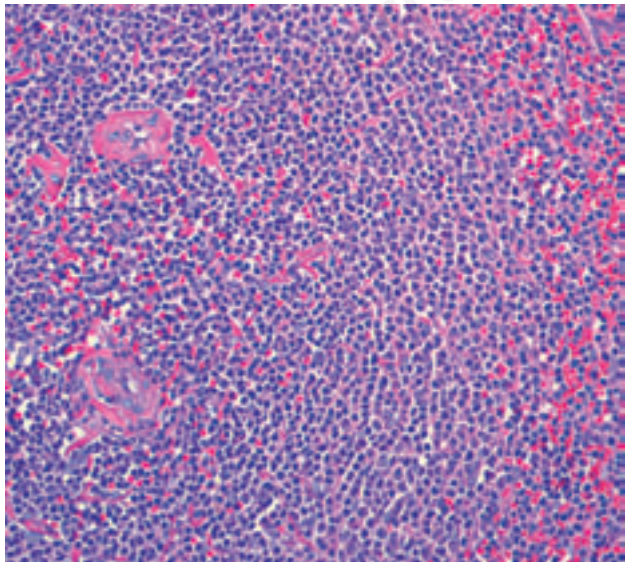

Figure 1. Hematoxylin-eosin stain (200x) of a case of splenic marginal zone lymphoma showing a white pulp nodule with two cytological features: small lymphocytes in the center and medium-sized pale cells at the periphery.

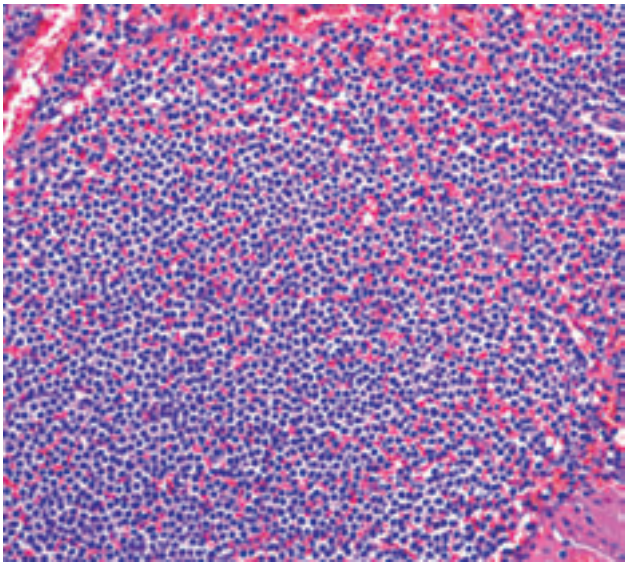

Figure 2. A white pulp nodule (hematoxylin-eosin stain, 200x) from a case of splenic marginal zone lymphoma displaying a uniform population composed mainly of medium-sized monocytoid cells with pale or eosinophilic cytoplasm.

teria for either of the provisional entities or for other better established B-cell lymphomas should be diagnosed as *splenic B-cell lymphoma/leukemia, unclassifiable* until more is known. These cases are characterized by isolated splenomegaly, without bone marrow, peripheral blood, or peripheral lymph node involvement. Spleen histology shows a micronodular pattern, without marginal zone differentiation. Some of the unclassifiable cases may belong to potential entities perhaps best defined by their cytogenetic abnormalities, such as BCL3 or CDK6 translocations.[25]

CD5-positive splenic marginal zone lymphoma

The intriguing report by Baseggio *et al.* of CD5-positive SMZL raises two questions: (i) is CD5-positive SMZL a phenotypic variant of SMZL with aberrant expression of CD5 or a separate clinico-pathological entity? (ii) how should classical SMZL and this new C5-positive subset be considered in relation to the heterogeneous spectrum of primary splenic small B-cell lymphomas?

Precise answers to these questions are still difficult. The term marginal zone lymphoma has often been used as a waste basket for any small B-cell splenic lymphomas that could not be otherwise categorized. To avoid lumping entities that could be biologically different under the definition of SMZL, the 2008 WHO classification opportunely created the category of *splenic B-cell lymphoma/leukemia, unclassifiable*. However, a proliferation of new splenic lymphoma entities in addition to classical SMZL, characterized by variant phenotypic expression and/or cytological features of peripheral blood, should be discouraged until reliable and reproducible diagnostic and genetic/molecular marker(s) for SMZL and its variants are identified. Strong collaboration among clinicians and pathologists is desirable: sharing and reviewing together clinical and histological data from large numbers of cases of primary splenic lymphomas could be useful for clarifying borderline cases of indolent splenic B-cell lymphoma and, also, for deciding a homogenous approach to the difficult task of diagnosis by means of only bone marrow histology combined with flow cytometry in the absence of spleen specimens.

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