Diagnosis of Splenic B-Cell Lymphomas in the Bone Marrow

A Review of Histopathologic, Immunophenotypic, and Genetic Findings

Amir Behdad, MD; Nathanael G. Bailey, MD

• Splenic B-cell lymphomas are a heterogeneous group of diseases comprising several entities that exhibit overlapping features. Diagnosis of these lymphomas has been reliant on the histopathologic examination of the spleen. However, with advances in diagnostic modalities and therapy, splenectomy is not commonly performed, and diagnosis and subclassification must be rendered based on the blood and bone marrow findings. In this brief review, we summarize the morphologic, immunophenotypic, and genetic findings of splenic B-cell lymphomas in the blood and bone marrow.

(*Arch Pathol Lab Med*. 2014;138:1295–1301; doi: 10.5858/arpa.2014-0291-CC)

Which lymphoma entities constitute the splenic lymphomas (SLs)? The spleen may be involved secondarily by virtually any type of systemic lymphoma originating in the lymph nodes or in an extranodal site; infrequently, lymphomas that are characteristically nodal or extranodal (large B-cell lymphomas, follicular lymphomas, and others) may be entirely restricted to the spleen at diagnosis.[1–3] In these scenarios, the spleen is involved by lymphoma, but these are not typically considered to be SLs. In addition, some T-cell neoplasms, particularly T-cell large granular lymphocytic leukemia and hepatosplenic T-cell lymphoma, characteristically have prominent splenic involvement but will not be considered in this review.

We will restrict our discussion to the following B-cell neoplasms that characteristically exhibit prominent splenomegaly without significant lymphadenopathy: splenic marginal zone lymphoma (SMZL), hairy cell leukemia (HCL), and unclassifiable SLs (including the provisional World Health Organization entities HCL variant [HCL-v] and splenic diffuse red pulp small B-cell lymphoma [SDRPL]).[4] Splenic lymphomas are almost never restricted to the spleen: they almost always exhibit blood and bone marrow involvement. This fact, along with advances in immunophenotypic techniques and the emergence of newly discovered genetic abnormalities, provides an opportunity for pathologists to diagnose these disorders based on the blood and bone marrow findings, with the caveat that some cases will remain difficult to definitively classify in the absence of a splenectomy specimen. The objective of this review is to summarize and compare the blood (Figure 1) and bone marrow (Figure 2) findings for each of these SLs and other lymphoid neoplasms that are included in the differential diagnosis. The immunophenotypic and genetic characteristics of SLs are summarized in Table 1 and Table 2, respectively.

SPLENIC MARGINAL ZONE LYMPHOMA

Splenic marginal zone lymphoma accounts for approximately 20% of all marginal zone lymphomas and 1% to 3% of non-Hodgkin lymphomas.[5] Most patients with SMZL are seen with splenomegaly, lymphocytosis, anemia, and thrombocytopenia. The cytopenias are due to hypersplenism or autoimmune phenomena. Epidemiological investigations have shown an association with hepatitis C infection in a subset of patients.[6]

Blood and Bone Marrow Morphology

Lymphoma cells are often present in the blood and demonstrate various degrees of heterogeneity, ranging from mature lymphocytes with abundant cytoplasm to medium-sized lymphoid cells with plasmacytoid features (Figure 1, A). The presence of large cells is not common and may represent progression or transformation. A subset of circulating lymphoma cells may demonstrate basophilic cytoplasm with short villi, often present at the poles of the cell. The bone marrow is almost always involved in SMZL, even in cases in which the leukemic component cannot be confirmed. Splenic marginal zone lymphoma demonstrates various patterns of infiltration in the bone marrow, typically with a nodular, interstitial, and intrasinusoidal distribution.[7] An anti-CD20 stain highlighting a mixed pattern of bone marrow involvement in a case of SMZL is shown in Figure 2, A.

Immunophenotype

Immunophenotyping assists diagnosis by confirming the presence of monoclonal B-cells that typically lack expression of surface CD5 and CD10. This phenotype, although nonspecific, is helpful to exclude other low-grade B-cell

Accepted for publication June 2, 2014.

From the Department of Pathology, University of Michigan Medical School, Ann Arbor.

The authors have no relevant financial interest in the products or companies described in this article.

Presented in part at New Frontiers in Pathology: An Update for Practicing Pathologists; September 26, 2013; Ann Arbor, Michigan.

Reprints: Nathanael G. Bailey, MD, Department of Pathology, University of Michigan Medical School, 1301 Catherine St, Mail Stop I5242, Ann Arbor, MI 48109 (e-mail: ngbailey@umich.edu).

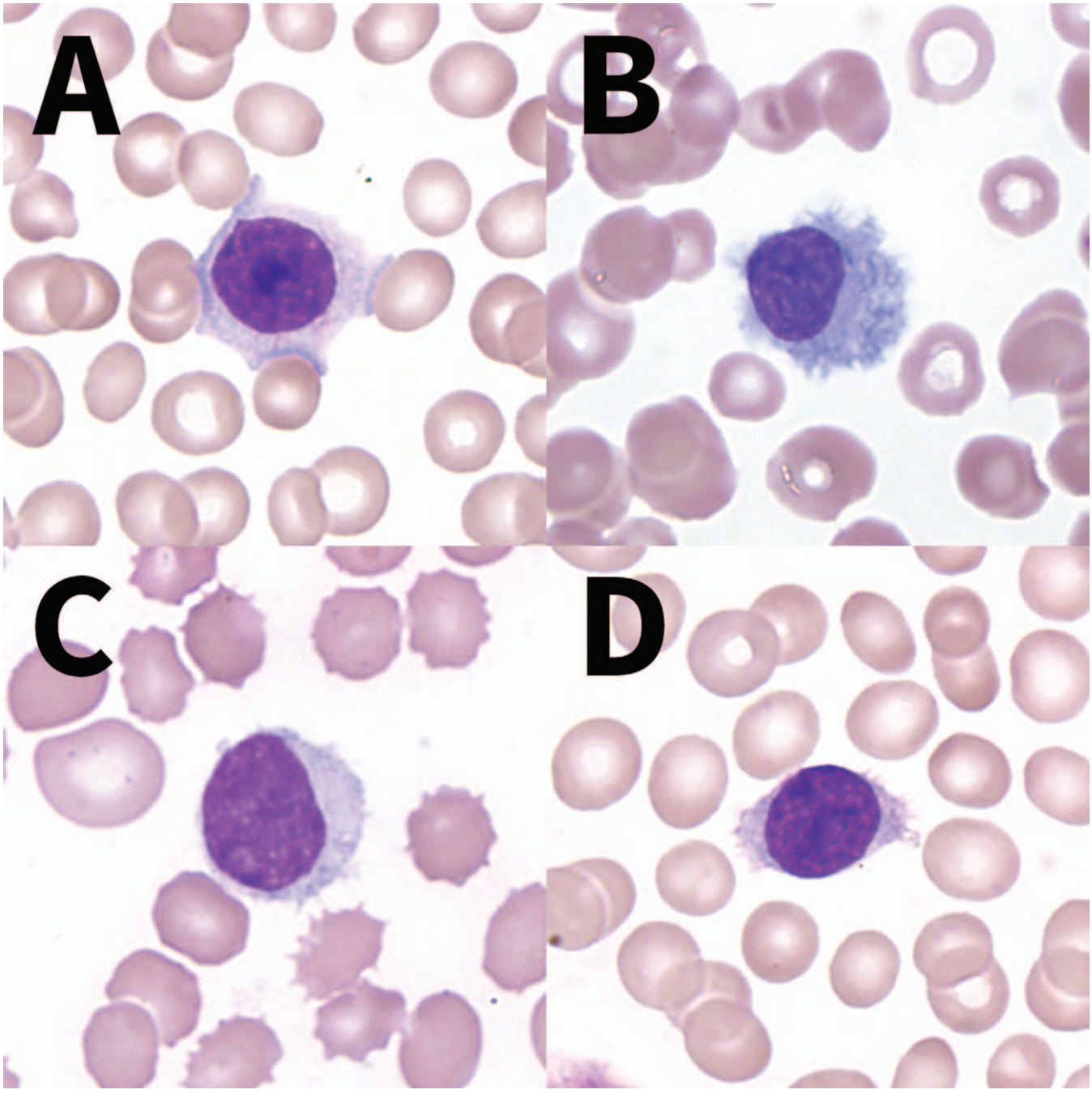

Figure 1. *Comparison of cytomorphologic findings (Wright-Giemsa stain, original magnifications ×1000). A, Splenic marginal zone lymphoma. Mature nuclear features are present, and many cells have abundant cytoplasm. Some villous lymphocytes may also be present. B, Hairy cell leukemia. Circumferential cytoplasmic villous projections are present. The nuclei may have oval or reniform shape with mature chromatin and without prominent nucleoli. C, Hairy cell leukemia variant. Hairy cytoplasmic projections are present, similar to hairy cell leukemia, but nucleoli are prominent. D, Splenic diffuse red pulp small B-cell lymphoma. Cytomorphologic findings can be similar to those of splenic marginal zone lymphoma; however, villous lymphocytes are reportedly more common.*

neoplasms such as follicular lymphoma ($CD10^+$), chronic lymphocytic leukemia/small lymphocytic lymphoma ($CD5^+$/$CD23^+$), and mantle cell lymphoma ($CD5^+$). However, an atypical phenotype does not rule out SMZL because 10% to 25% of SMZLs may express surface CD5.[7,8] In most cases, IgM with or without IgD is strongly expressed,[9] and a subset of neoplastic cells may express DBA.44, CD11c, CD23, CD103, or CD25.[10] Most important, SMZL virtually never exhibits strong coexpression of CD103, CD11c, and CD25 (a feature characteristic of HCL).[11–13]

Genetics

Most SMZLs harbor cytogenetic abnormalities. Deletion in the long arm of chromosome 7 (7q31) is the most common single cytogenetic aberrancy, followed by chromosome 3 gains.[14] Until recently, little was known about the individual gene mutations that underlie the pathogenesis of

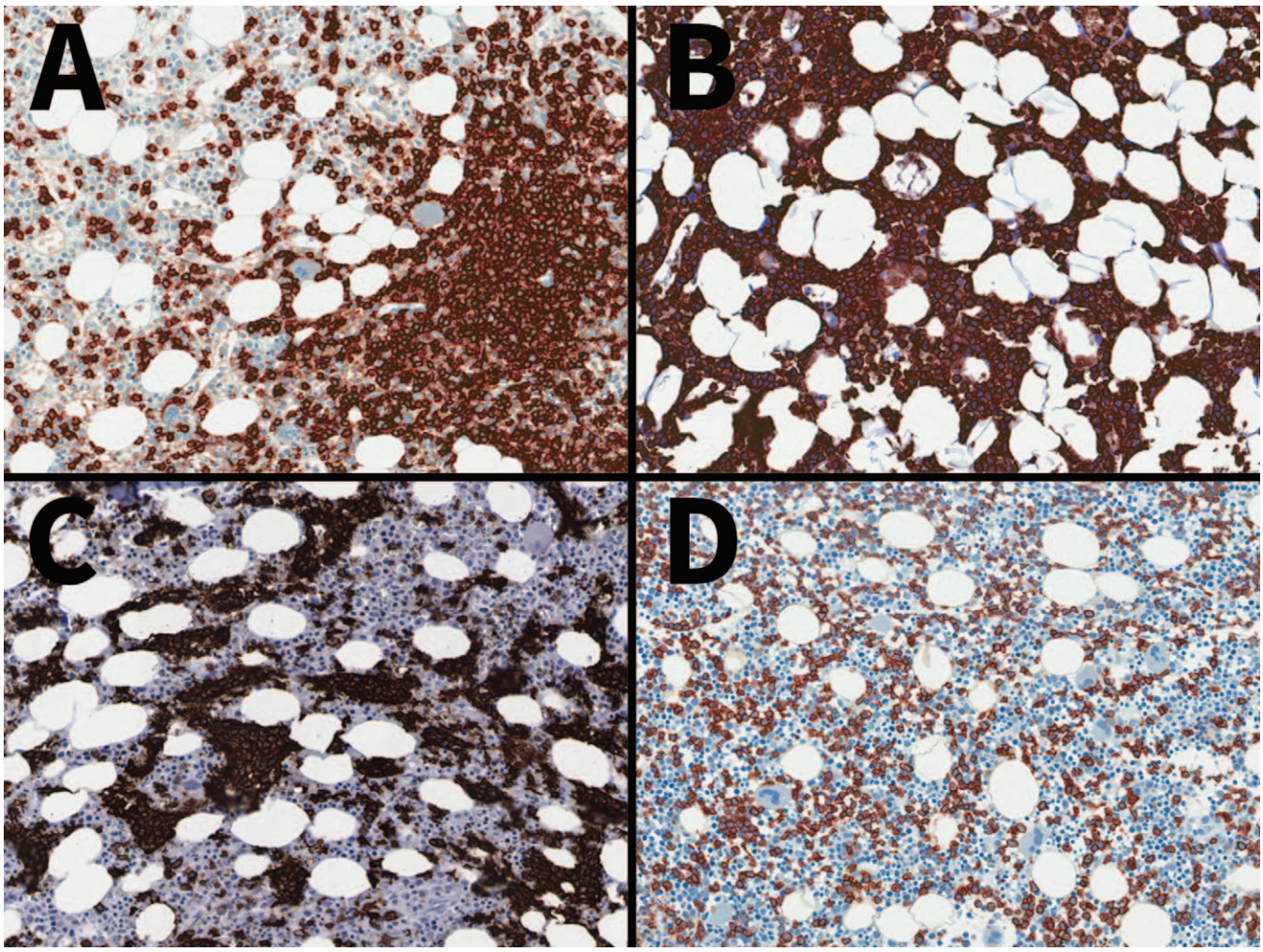

Figure 2. *Comparison of patterns of bone marrow involvement (anti-CD20 immunohistochemical staining, original magnifications ×200). A, Splenic marginal zone lymphoma typically exhibits nodular and interstitial marrow involvement, and intrasinusoidal spread may be seen. B, Hairy cell leukemia characteristically exhibits an interstitial or patchy pattern of infiltration. C, Hairy cell leukemia variant often exhibits a marked sinusoidal distribution, as seen in this case, but interstitial involvement similar to that of hairy cell leukemia may also be seen. D, Splenic diffuse red pulp small B-cell lymphoma may exhibit intrasinusoidal, interstitial, or nodular distribution.*

SMZL. Somatic gain-of-function mutations in *NOTCH2* have been described in approximately 25% of SMZLs, and other NOTCH pathway genes are mutated in an additional 10%.[15,16] NOTCH2 signaling is necessary for the development of normal marginal zone B-cells[17,18]; the mutations identified in SMZL lead to stabilization of the NOTCH2 protein and increased NOTCH signaling.[19] Mutations and copy number abnormalities of nuclear factor–κB pathway genes are present in approximately one-third of cases,[16,20] and *TP53* deletions/mutations occur.[14,16,21] *NOTCH2* mutations and *TP53* alterations have been associated with adverse clinical outcomes in SMZL.[14,15,21,22]

Treatment

Management of patients with SMZL depends on the disease status, the severity of the clinical presentation, and the presence of other comorbidities. Asymptomatic patients are often followed up with a watch-and-wait policy. In

Table 1. Comparison of Immunophenotypic Findings in Splenic Small B-Cell Lymphomas[a]

Variable	CD5	CD10	CD11c	CD25	CD103	CD123	IgM	IgD	IgG	Annexin A1	DBA.44
SMZL	−/+	−	+/−	−/+	−/+	−	+	+/−	−/+	−	+/−
SDRPL	−/+	−	+	−	−/+	−/+	+/−	+/−	+/−	−	+
HCL-v	−	−	+	−	+	−	−/+	−/+	+/−	−	+
HCL	−	−/+	+	+	+	+	+	+	+	+	+
LPL	−/+	−	+	+/−	−	−	+	−	−	−	−

Abbreviations: HCL, hairy cell leukemia; HCL-v, hairy cell leukemia variant; LPL, lymphoplasmacytic lymphoma; SDRPL, splenic diffuse red pulp small B-cell lymphoma; SMZL, splenic marginal zone lymphoma; +, positive in more than 75% of cases; +/−, positive in more than 50% to 75% of cases; −/+, positive in 10% to 50% of cases; −, negative in more than 90% of cases.

[a] Data are aggregated from references 7, 9, 10, 12, 13, 25, 37, 46, 47, 53, 58–62.

Table 2. Comparison of Genetic Aberrations in Splenic Small B-Cell Lymphomas[a]

Variable	Gene Mutation	Associated Structural Abnormality
SMZL	*NOTCH2* (~25%) NF-κB pathway genes (*BIRC3*, *TNFAIP3*, *MAP3K14*, *IKBKB*) (~33%) Rare: *MYD88* L265P	del 7q (~45%); Less commonly: trisomy 3, trisomy 12, trisomy 18, 17p (*TP53*)
SDRPL	Unknown	Uncommon: del 7q, trisomy 18, del 17p (*TP53*)
HCL-v	*MAP2K1* (~33%)	del 17p (*TP53*) (~33%); Uncommon: 5q gain, del 7q
HCL	*BRAF* V600E (>90%)	Rare: 5q gain, del 7q
LPL	*MYD88* L265P (~90%)	del 6q (~45%); Uncommon: del 13q, del 7q

Abbreviations: HCL, hairy cell leukemia; HCL-v, hairy cell leukemia variant; LPL, lymphoplasmacytic lymphoma; NF-κB, nuclear factor–κB; SDRPL, splenic diffuse red pulp small B-cell lymphoma; SMZL, splenic marginal zone lymphoma.

[a] Data are aggregated from references 14–16, 26, 40, 47, 50, 63–65.

patients with hepatitis C virus infection, treatment of the viral infection may cause regression of the lymphoma.[6] In symptomatic patients with no history of hepatitis C virus infection, several options are available, including splenectomy, rituximab monotherapy, and immunochemotherapy.[23,24]

HAIRY CELL LEUKEMIA

Hairy cell leukemia is a lymphoid neoplasm that is characterized by diffuse infiltration of the spleen and bone marrow, leading to cytopenias and splenomegaly. The identification of HCL, and the discrimination from other SLs, is critical because the therapeutic approach for HCL is distinct from that of most other B-cell neoplasms.

Blood and Bone Marrow Morphology

Patients with HCL are often pancytopenic, and monocytopenia is a characteristic finding. Typically, the neoplastic B-cells can be identified in the blood; however, they are sometimes few in number and fairly subtle morphologically. Typical hairy cells (Figure 1, B) have round to reniform nuclei without prominent nucleoli and with a moderate amount of pale cytoplasm. The periphery of the cytoplasm exhibits the circumferential, characteristic villiform projections that lend the disease its name. The bone marrow contains an interstitial or diffuse infiltrate of lymphocytes with abundant, pale cytoplasm (Figure 2, B). In some cases, the bone marrow is profoundly hypocellular, so the exclusion of subtle HCL infiltrates should be performed before a diagnosis of bone marrow aplasia in adults.[12]

Immunophenotype

A diagnosis of HCL is typically confirmed by the identification of a characteristic immunophenotype. Hairy cell leukemia expresses bright pan–B-cell antigens and, uniquely among B-cell neoplasms, strongly expresses CD11c, CD25, CD103, CD123, and annexin A1 typically.[12,13,25] This characteristic immunophenotype is highly sensitive and specific for HCL.

Genetics

Recently, activating *BRAF* V600E mutations were identified in classic HCL, occurring in all HCL cases in the initial study[26] and in an early subsequent study.[27] Although the *BRAF* V600E mutation is commonly present in various neoplasms (colorectal cancer, melanoma, papillary thyroid cancer, Langerhans cell histiocytosis, non–small cell lung cancer, and others), among lymphomas it seems highly specific for HCL.[12,26–29] Although most HCLs are *BRAF* mutated, a subset of patients with phenotypically classic HCL lack *BRAF* mutations,[29] exhibit unmutated *IGHV4-34* gene use, and have poor responses to conventional therapy.[30]

Treatment

Asymptomatic patients with HCL may be followed up expectantly. When treatment is required, the initial approach to HCL is substantially different from that of other B-cell malignancies. The initial therapy typically consists of single-agent purine analogues such as pentostatin or cladribine, which yield durable responses in most patients.[31–33] Although these standard therapies are generally well tolerated and highly effective in HCL, some patients develop refractory disease. Various therapeutic approaches exist in this setting,[31] and several recent case reports suggest that the BRAF inhibitor vemurafenib elicits dramatic responses in *BRAF*-mutated refractory HCL.[34–36] The long-term efficacy and appropriate dose schedule for vemurafenib still remains to be established in HCL.

UNCLASSIFIABLE SLs

HCL Variant

Hairy cell leukemia variant is a provisional entity in the World Health Organization classification.[4] As the name implies, HCL-v exhibits some, but not all, of the features of classic HCL, including hairlike cytoplasmic projections.

Blood and Bone Marrow Morphology.—In contrast to HCL, which typically is seen with cytopenias and rare circulating hairy cells, HCL-v often manifests lymphocytosis, and the characteristic monocytopenia observed in HCL is usually not present in HCL-v. Hairy cell leukemia variant cells (Figure 1, C) frequently have prominent nucleoli (prolymphocytic morphology), which is not a feature of typical HCL.[37] Hairy cell leukemia variant may tend to exhibit less extensive marrow involvement than HCL and can have interstitial, predominantly sinusoidal, or diffuse patterns of marrow infiltration (Figure 2, C).[12,38,39]

Immunophenotype.—Much like HCL, HCL-v usually expresses bright pan–B-cell antigens, along with CD103 and CD11c. However, HCL-v typically lacks expression of CD25, CD123, and annexin A1, which are all almost always expressed in classic HCL.[12,25]

Genetics.—Multiple studies[12,26–29] have confirmed that HCL-v lacks the *BRAF* V600E mutation that is characteristic of HCL. However, downstream mutations of *MAP2K1* (*MEK1*) have been identified in approximately one-third of HCL-vs and in most *IGHV4-34*–expressing classic HCLs.[40] These findings unify to some extent the pathobiology of HCL and HCL-v because both disorders frequently exhibit activation of the RAS-RAF-MAPK signaling cascade. *TP53* deletions seem to be frequent events in HCL-v and could contribute to its more aggressive disease course compared with HCL.[41]

Treatment.—Distinction between HCL and HCL-v has important clinical implications because HCL-v does not respond well to standard HCL single-agent purine analogue therapy (eg, cladribine and pentostatin).[42] Optimal therapy for HCL-v is not established, but some good outcomes have been reported with the addition of rituximab to cladribine therapy.[43] The identification of *MAP2K1* mutations raises the possibility of specific inhibition of the MAPK pathway as a novel therapeutic strategy in HCL-v.

Splenic Diffuse Red Pulp Small B-Cell Lymphoma

Splenic diffuse red pulp small B-cell lymphoma is rare and is another provisional entity within the category of splenic B-cell lymphoma/leukemia unclassifiable.[4] The features of this disorder seem to overlap significantly with the entity originally described as splenic lymphoma with circulating villous lymphocytes, as defined by the French-American-British classification.[44,45] However, splenic lymphoma with circulating villous lymphocytes has also been considered to be largely synonymous with SMZL, and precise discrimination of these disorders is challenging. Splenic diffuse red pulp small B-cell lymphoma is uncommon, and so far only a few case series have described the clinicopathologic findings.[46,47] Similar to other splenic B-cell lymphomas, patients often have splenomegaly, moderate lymphocytosis, and variable cytopenias.

Blood and Bone Marrow Morphology.—As the splenic lymphoma with circulating villous lymphocytes terminology implies, circulating lymphoma cells often demonstrate characteristic cytoplasmic villous projections, along with basophilic cytoplasm (Figure 1, D).[46,47] It is important to once again note that cytoplasmic projections are not specific to a particular type of lymphoma, and, as described previously, they can be seen in SMZL, HCL, and HCL-v, as well as other low-grade B-cell neoplasms. Bone marrow involvement has been seen in all reported cases, and the lymphoma seems to show a variable interstitial and sinusoidal pattern of infiltration (Figure 2, D).[7,46,47]

Immunophenotype.—The immunophenotype of SDRPL is typically that of a $CD5^-$, $CD10^-$, mature B-cell neoplasm. Similar to HCL-v and unlike most cases of SMZL, SDRPL cells frequently express DBA.44 and IgG; they are less likely to express CD103 than HCL-v, and CD27 is not expressed as frequently as it is in SMZL.[7,46,47]

Genetics.—No definitive recurrent cytogenetic or molecular aberration has been established in this lymphoma. Most studied cases have had normal karyotypes; however, the abnormal cases have abnormalities that overlap with those seen in other SLs such as deletions of 7q and *TP53* alterations.[46–48] *BRAF* mutations have not been reported.

Treatment.—The clinical course of this provisional entity seems to be indolent and even more favorable than that of SMZL.[46] The discrimination between SDRPL and SMZL may not be possible on the basis of only the bone marrow and blood findings[7]; however, therapeutic strategies for SDRPL do not differ from those of SMZL, and splenectomy solely to resolve uncertainty in classification is not suggested.[24] Other splenic small B-cell lymphomas not fulfilling the criteria for HCL-v or SDRPL should be diagnosed as splenic B-cell lymphoma, unclassifiable.

Other B-Cell Lymphomas Involving Blood and Bone Marrow Mimicking SLs

Several other B-cell neoplasms can have overlapping features with the SLs and may exhibit involvement of the blood, bone marrow, and spleen.

Lymphoplasmacytic lymphoma (LPL) is often in the differential diagnosis with SMZL because SMZL may exhibit plasmacytic differentiation, and LPL may sometimes be associated with splenomegaly. The LPL infiltrate in the bone marrow contains variable proportions of lymphocytes, plasmacytoid lymphocytes, and plasma cells. The pattern of infiltration in the bone marrow can be diffuse, interstitial, or nodular. The immunophenotype, similar to SMZL, is typically that of a $CD5^-$/$CD10^-$ mature B-cell neoplasm.

Detection of monoclonal IgM in the serum is typical in LPL, and monoclonal serum IgM combined with the presence of LPL in the bone marrow defines the clinicopathologic entity of Waldenström macroglobulinemia.[49] However, marginal zone lymphomas and other B-cell lymphoproliferative disorders may be associated with an IgM paraprotein, so this finding alone is not fully discriminatory. Recently, somatic mutations in *MYD88* were described in a high proportion of LPL cases.[50] *MYD88* mutations are infrequent in SMZL and other low-grade B-cell lymphomas,[51,52] and detection of a *MYD88* mutation is a useful diagnostic adjunct in the appropriate clinical setting.[53]

B-cell prolymphocytic leukemia (B-PLL) is a somewhat ill-defined, uncommon entity characterized by marked peripheral lymphocytosis composed of more than 55% prolymphocytes (large lymphocytes with a prominent central nucleolus). Splenomegaly and bone marrow involvement are common.[4] Many cases of putative B-PLL have *TP53* deletions,[54] and B-PLL is associated with an aggressive disease course. Historically, cases diagnosed as B-PLL have represented a heterogeneous group of diseases: older literature includes cases that now would be considered to be transformed chronic lymphocytic leukemia/small lymphocytic lymphoma and mantle cell lymphoma.[55,56] It has also been recognized that some cases of B-PLL are associated with antecedent SMZL, suggesting that many of the remaining cases in the B-PLL category represent transformed SLs.[57] It remains unclear whether B-PLL should be retained as a distinct diagnostic entity.

Other low-grade B-cell lymphomas such as chronic lymphocytic leukemia/small lymphocytic lymphoma, mantle cell lymphoma, non-SMZLs, and follicular lymphomas may also be included in the differential diagnosis with the SLs. However, typically the immunophenotype, morphology, and other clinical findings help to easily distinguish these entities. Details of the findings in these neoplasms are beyond the scope of this review.

CONCLUSIONS

Morphologic examination and immunophenotyping of blood and bone marrow enable diagnosis of SLs in most patients, without the need for splenectomy. Recognizing HCL, and distinguishing it from HCL-v and other SLs, is critical because of substantially different therapeutic approaches. The genetic basis of SLs is beginning to be uncovered, and genetic testing may contribute to diagnosis in select cases and identify targets for precision therapy.

References

1. Howard MT, Dufresne S, Swerdlow SH, Cook JR. Follicular lymphoma of the spleen: multiparameter analysis of 16 cases. *Am J Clin Pathol*. 2009;131(5):656–662.

2. Mollejo M, Rodriguez-Pinilla MS, Montes-Moreno S, et al. Splenic follicular lymphoma: clinicopathologic characteristics of a series of 32 cases. *Am J Surg Pathol*. 2009;33(5):730–738.

3. Warnke RA, Weiss LM, Chan JK, Cleary ML, Dorfman RF. Lymphomas of the spleen. In: *Tumors of the Lymph Nodes and Spleen*. Washington, DC: Armed Forces Institute of Pathology; 1995:411–434.

4. Swerdlow SH; International Agency for Research on Cancer, World Health Organization. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: International Agency for Research on Cancer; 2008.

5. Berger F, Felman P, Thieblemont C, et al. Non-MALT marginal zone B-cell lymphomas: a description of clinical presentation and outcome in 124 patients. *Blood*. 2000;95(6):1950–1956.

6. Hermine O, Lefrere F, Bronowicki JP, et. al. Regression of splenic lymphoma with villous lymphocytes after treatment of hepatitis C virus infection. *N Engl J Med*. 2002;347(2):89–94.

7. Ponzoni M, Kanellis G, Pouliou E, et al. Bone marrow histopathology in the diagnostic evaluation of splenic marginal-zone and splenic diffuse red pulp small B-cell lymphoma: a reliable substitute for spleen histopathology? *Am J Surg Pathol*. 2012;36(11):1609–1618.

8. Baseggio L, Traverse-Glehen A, Petinataud F, et al. CD5 expression identifies a subset of splenic marginal zone lymphomas with higher lymphocytosis: a clinico-pathological, cytogenetic and molecular study of 24 cases. *Haematologica*. 2009;95(4):604–612.

9. Matutes E, Morilla R, Owusu-Ankomah K, et al. The immunophenotype of splenic lymphoma with villous lymphocytes and its relevance to the differential diagnosis with other B-cell disorders. *Blood*. 1994;83(6):1558–1562.

10. Matutes E, Oscier D, Montalban C, et al. Splenic marginal zone lymphoma proposals for a revision of diagnostic, staging and therapeutic criteria. *Leukemia*. 2008;22(3):487–495.

11. Dong HY, Weisberger J, Liu Z, Tugulea S. Immunophenotypic analysis of CD103$^+$ B-lymphoproliferative disorders: hairy cell leukemia and its mimics. *Am J Clin Pathol*. 2009;131(4):586–595.

12. Shao H, Calvo KR, Grönborg M, et al. Distinguishing hairy cell leukemia variant from hairy cell leukemia: development and validation of diagnostic criteria. *Leuk Res*. 2013;37(4):401–409.

13. Venkataraman G, Aguhar C, Kreitman RJ, et al. Characteristic CD103 and CD123 expression pattern defines hairy cell leukemia: usefulness of CD123 and CD103 in the diagnosis of mature B-cell lymphoproliferative disorders. *Am J Clin Pathol*. 2011;136(4):625–630.

14. Salido M, Baró C, Oscier D, et al. Cytogenetic aberrations and their prognostic value in a series of 330 splenic marginal zone B-cell lymphomas: a multicenter study of the Splenic B-cell Lymphoma Group. *Blood*. 2010;116(9):1479–1488.

15. Kiel MJ, Velusamy T, Betz BL, et al. Whole-genome sequencing identifies recurrent somatic *NOTCH2* mutations in splenic marginal zone lymphoma. *J Exp Med*. 2012;209(9):1553–1565.

16. Rossi D, Trifonov V, Fangazio M, et al. The coding genome of splenic marginal zone lymphoma: activation of *NOTCH2* and other pathways regulating marginal zone development. *J Exp Med*. 2012;209(9):1537–1551.

17. Saito T, Chiba S, Ichikawa M, et al. Notch2 is preferentially expressed in mature B cells and indispensable for marginal zone B lineage development. *Immunity*. 2003;18(5):675–685.

18. Witt CM, Won WJ, Hurez V, Klug CA. Notch2 haploinsufficiency results in diminished B1 B cells and a severe reduction in marginal zone B cells. *J Immunol*. 2003;171(6):2783–2788.

19. Puente XS, Pinyol M, Quesada V, et al. Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. *Nature*. 2011;475(7354):101–105.

20. Rossi D, Deaglio S, Dominguez-Sola D, et al. Alteration of *BIRC3* and multiple other NF-κB pathway genes in splenic marginal zone lymphoma. *Blood*. 2011;118(18):4930–4934.

21. Rinaldi A, Mian M, Chigrinova E, et al. Genome-wide DNA profiling of marginal zone lymphomas identifies subtype-specific lesions with an impact on the clinical outcome. *Blood*. 2011;117(5):1595–1604.

22. Algrin C, Brisou G, Beldjord K, et al. Prognostic impact of somatic *NOTCH2* mutation in splenic marginal zone lymphoma [abstract]. *Blood*. 2013;122(21):4247. http://bloodjournal.hematologylibrary.org/content/122/21/4247.short. Accessed June 16, 2014.

23. Kalpadakis C, Pangalis GA, Vassilakopoulos TP, Sachanas S, Angelopoulou MK. Treatment of splenic marginal zone lymphoma: should splenectomy be abandoned [published online ahead of print November 6, 2013]? *Leuk Lymphoma*. doi:10.3109/10428194.2013.845884.

24. Iannitto E, Tripodo C. How I diagnose and treat splenic lymphomas. *Blood*. 2011;117(9):2585–2595.

25. Falini B, Tiacci E, Liso A, et al. Simple diagnostic assay for hairy cell leukaemia by immunocytochemical detection of annexin A1 (ANXA1). *Lancet*. 2004;363(9424):1869–1871.

26. Tiacci E, Trifonov V, Schiavoni G, et al. *BRAF* mutations in hairy cell leukemia. *N Engl J Med*. 2011;364(24):2305–2315.

27. Boyd EM, Bench AJ, van't Veer MB, et al. High resolution melting analysis for detection of *BRAF* exon 15 mutations in hairy cell leukaemia and other lymphoid malignancies. *Br J Haematol*. 2011;155(5):609–612.

28. Laurini JA, Aoun P, Iqbal J, et al. Investigation of the *BRAF* V600E mutation by pyrosequencing in lymphoproliferative disorders. *Am J Clin Pathol*. 2012;138(6):877–883.

29. Xi L, Arons E, Navarro W, et al. Both variant and IGHV4-34–expressing hairy cell leukemia lack the *BRAF* V600E mutation. *Blood*. 2012;119(14):3330–3332.

30. Arons E, Suntum T, Stetler-Stevenson M, Kreitman RJ. VH4-34$^+$ hairy cell leukemia, a new variant with poor prognosis despite standard therapy. *Blood*. 2009;114(21):4687–4695.

31. Grever MR. How I treat hairy cell leukemia. *Blood*. 2010;115(1):21–28.

32. Piro LD, Carrera CJ, Carson DA, Beutler E. Lasting remissions in hairy-cell leukemia induced by a single infusion of 2-chlorodeoxyadenosine. *N Engl J Med*. 1990;322(16):1117–1121.

33. Spiers AS, Parekh SJ, Bishop MB. Hairy-cell leukemia: induction of complete remission with pentostatin (2′-deoxycoformycin). *J Clin Oncol*. 1984;2(12):1336–1342.

34. Dietrich S, Hüllein J, Hundemer M, et al. Continued response off treatment after BRAF inhibition in refractory hairy cell leukemia. *J Clin Oncol*. 2013;31(19):e300–e303. doi:10.1200/JCO.2012.45.9495.

35. Follows GA, Sims H, Bloxham DM, et al. Rapid response of biallelic *BRAF* V600E mutated hairy cell leukaemia to low dose vemurafenib. *Br J Haematol*. 2013;161(1):150–153.

36. Munoz J, Schlette E, Kurzrock R. Rapid response to vemurafenib in a heavily pretreated patient with hairy cell leukemia and a *BRAF* mutation. *J Clin Oncol*. 2013;31(20):e351–e352. doi:10.1200/JCO.2012.45.7739.

37. Sainati L, Matutes E, Mulligan S, et al. A variant form of hairy cell leukemia resistant to alpha-interferon: clinical and phenotypic characteristics of 17 patients. *Blood*. 1990;76(1):157–162.

38. Cessna MH, Hartung L, Tripp S, et al. Hairy cell leukemia variant: fact or fiction? *Am J Clin Pathol*. 2005;123(1):132–138.

39. Matutes E, Wotherspoon A, Catovsky D. The variant form of hairy-cell leukaemia. *Best Pract Res Clin Haematol*. 2003;16(1):41–56.

40. Waterfall JJ, Arons E, Walker RL, et al. High prevalence of *MAP2K1* mutations in variant and IGHV4-34–expressing hairy-cell leukemias. *Nat Genet*. 2014;46(1):8–10.

41. Vallianatou K, Brito-Babapulle V, Matutes E, Atkinson S, Catovsky D. p53 Gene deletion and trisomy 12 in hairy cell leukemia and its variant. *Leuk Res*. 1999;23(11):1041–1045.

42. Robak T, Błasińska-Morawiec M, Błasiński J, et al. 2-Chlorodeoxyadenosine (cladribine) in the treatment of hairy cell leukemia and hairy cell leukemia variant: 7-year experience in Poland. *Eur J Haematol*. 1999;62(1):49–56.

43. Ravandi F, O'Brien S, Jorgensen J, et al. Phase 2 study of cladribine followed by rituximab in patients with hairy cell leukemia. *Blood*. 2011;118(14):3818–3823.

44. Bennett JM, Catovsky D, Daniel MT, et al; French-American-British (FAB) Cooperative Group. Proposals for the classification of chronic (mature) B and T lymphoid leukaemias. *J Clin Pathol*. 1989;42(6):567–584.

45. Traverse-Glehen A, Baseggio L, Salles G, oiffier B, Felman P, Berger F. Splenic diffuse red pulp small B cell lymphoma: toward the emergence of a new lymphoma entity. *Discov Med*. 2012;13(71):253–265.

46. Kanellis G, Mollejo M, Montes-Moreno S, et al. Splenic diffuse red pulp small B-cell lymphoma: revision of a series of cases reveals characteristic clinicopathological features. *Haematologica*. 2010;95(7):1122–1129.

47. Traverse-Glehen A, Baseggio L, Bauchu EC, et al. Splenic red pulp lymphoma with numerous basophilic villous lymphocytes: a distinct clinicopathologic and molecular entity? *Blood*. 2008;111(4):2253–2260.

48. Mollejo M, Algara P, Mateo MS, et al. Splenic small B-cell lymphoma with predominant red pulp involvement: a diffuse variant of splenic marginal zone lymphoma? *Histopathology*. 2002;40(1):22–30.

49. Owen RG, Treon SP, Al-Katib A, et al. Clinicopathological definition of Waldenström's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenström's Macroglobulinemia. *Semin Oncol*. 2003;30(2):110–115.

50. Treon SP, Xu L, Yang G, et al. MYD88 L265P somatic mutation in Waldenström's macroglobulinemia. *N Engl J Med*. 2012;367(9):826–833.

51. Martínez N, Almaraz C, Vaqué JP, et al. Whole-exome sequencing in splenic marginal zone lymphoma reveals mutations in genes involved in marginal zone differentiation. *Leukemia*. 2014;28(6):1334–1340.

52. Varettoni M, Arcaini L, Zibellini S, et al. Prevalence and clinical significance of the *MYD88* (L265P) somatic mutation in Waldenström's macroglobulinemia and related lymphoid neoplasms. *Blood*. 2013;121(13):2522–2528.

53. Konoplev S, Medeiros LJ, Bueso-Ramos CE, et al. Immunophenotypic profile of lymphoplasmacytic lymphoma/Waldenström macroglobulinemia.*Am J Clin Pathol*. 2005;124(3):414–420.

54. Del Giudice I, Davis Z, Matutes E, et al. IgVH genes mutation and usage, ZAP-70 and CD38 expression provide new insights on B-cell prolymphocytic leukemia (B-PLL). *Leukemia*. 2006;20(7):1231–1237.

55. Enno A, Catovsky D, O'Brien M, et al. "Prolymphocytoid" transformation of chronic lymphocytic leukaemia. *Br J Haematol*. 1979;41(1):9–18.

56. Schlette E, Bueso-Ramos C, Giles F, et al. Mature B-cell leukemias with more than 55% prolymphocytes: a heterogeneous group that includes an unusual variant of mantle cell lymphoma. *Am J Clin Pathol*. 2001;115(4):571–581.

57. Hoehn D, Miranda RN, Kanagal-Shamanna R, et al. Splenic B-cell lymphomas with more than 55% prolymphocytes in blood: evidence for prolymphocytoid transformation. *Hum Pathol*. 2012;43(11):1828–1838.

58. Chen YH, Tallman MS, Goolsby C, Peterson L. Immunophenotypic variations in hairy cell leukemia.*Am J Clin Pathol*. 2006;125(2):251–259.

59. Forconi F, Sahota SS, Raspadori D, et al. Hairy cell leukemia: at the crossroad of somatic mutation and isotype switch. *Blood*. 2004;104(10):3312–3317.

60. Hunter ZR, Xu L, Yang G, et al. The genomic landscape of Waldenström macroglobulinemia is characterized by highly recurring *MYD88* and WHIM-like *CXCR4* mutations, and small somatic deletions associated with B-cell lymphomagenesis. *Blood*. 2014;123(11):1637–1646.

61. Morice WG, Chen D, Kurtin PJ, et al. Novel immunophenotypic features of marrow lymphoplasmacytic lymphoma and correlation with Waldenström's macroglobulinemia. *Mod Pathol*. 2009;22(6):807–816.

62. Went PT, Zimpfer A, Pehrs AC, et al. High specificity of combined TRAP and DBA.44 expression for hairy cell leukemia. *Am J Surg Pathol*. 2005;29(4):474–478.

63. Braggio E, Dogan A, Keats JJ, et al. Genomic analysis of marginal zone and lymphoplasmacytic lymphomas identified common and disease-specific abnormalities. *Mod Pathol*. 2012;25(5):651–660.

64. Hockley SL, Morgan GJ, Leone PE, et al. High-resolution genomic profiling in hairy cell leukemia–variant compared with typical hairy cell leukemia. *Leukemia*. 2011;25(7):1189–1192.

65. Traverse-Glehen A, Bachy E, Baseggio L, et al. Immunoarchitectural patterns in splenic marginal zone lymphoma: correlations with chromosomal aberrations, *IGHV* mutations, and survival: a study of 76 cases. *Histopathology*. 2013;62(6):876–893.