Histopathological and Immunohistochemical Features of 32 Cases of Splenic B-Cell Lymphoma and Leukemia

Otuz İki Dalak Lenfoma ve Lösemi Olgusunun Histopatolojik ve İmmünohistokimyasal Özellikleri

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ABSTRACT Objective: Leukemias and non-Hodgkin lymphomas commonly involve the spleen or originate primarily in the spleen and then spread to other sites. **Material and Methods:** In this retrospective study, we examined the histopathological and immunohistochemical characteristics of 32 cases of primary or secondary splenic B-cell lymphoma and leukemia, in which the diagnosis was established according to the World Health Organization (WHO) classification. The immunohistochemical panel included ALK-1, BCL-2, BCL-6, CD3, CD5, CD10, CD20, CD21, CD23, CD30, CD43, cyclin D1, Ki-67, and TRAP. **Results:** There was no other nodal or extranodal disease involvement in the majority of patients diagnosed with lymphoma at the time of presentation, while cases of leukemia had undergone splenectomy for palliative purposes. The diagnoses were as follows: 11 cases of hairy cell leukemia (HCL, 34.4%), 8 cases of splenic marginal zone lymphoma (SMZL, 25%), 8 cases of diffuse large B-cell lymphoma (DLBCL, 25%) including 1 T-cell-rich B-cell lymphoma (TCRBCL), 4 cases of mantle cell lymphoma (MCL, 12.5%), and 1 prolymphocytic leukemia (PLL, 3.1%). **Conclusion:** Overall assessment of spleen, liver, bone marrow, and lymph node examinations and a detailed correlation of the histopathological and immunohistochemical features with the clinical findings are very helpful and usually lead to the final diagnosis in most cases of primary or secondary splenic B-cell lymphoma and leukemia.

Key Words: Lymphoma, non-Hodgkin; splenic neoplasms; leukemia, B-cell

ÖZET Amaç: Lösemi ve non-Hodkgin lenfomalar sıklıkla dalağı tutar ya da primer olarak dalak kökenli başlayıp daha sonra diğer alanlara yayılım yaparlar. **Gereç ve Yöntemler:** Bu retrospektif çalışmada, Dünya Sağlık Örgütü (DSÖ) sınıflama sistemi temel alınarak, primer ya da sekonder 32 splenik B-hücreli lenfoma ve lösemi olgusunun histopatolojik ve immünohistokimyasal özellikleri araştırılmıştır. Tüm olgulara, ALK-1, BCL-2, BCL-6, CD3, CD5, CD10, CD20, CD21, CD23, CD30, CD43, cyclin D1, Ki-67 ve TRAP dahil olacak şekilde immünohistokimyasal panel uygulanmıştır. **Bulgular:** Bu makale yazıldığı sırada (2004), splenik primer ya da sekonder lenfoma tanısı alan hastaların büyük bir kısmında, klinik ya da patolojik incelemeler sonucunda nodal ya da ekstranodal hastalık yayılımı veya tutulumu saptanmamıştır. Bu önemli bulgu, bu olgulara palyatif amaçlarla splenektomi operasyonu yapıldığını göstermektedir. **Tanılar:** 32 (%100) olgunun 11 (%34.4)'i saçlı hücreli lösemi, 8 (%25)'i splenik marjinal zon lenfoması, bir adet T-hücreden zengin B-hücreli lenfoma da dahil olacak şekilde 8 (%25)'i diffüz büyük hücreli lenfoma, 4 (%12.5)'ü mantle hücreli lenfoma ve 1 (%3.1)'i prolenfositik lösemi olarak tanımlanmıştır. **Sonuç:** Primer ya da sekonder splenik B-hücreli lenfoma veya lösemi tanısında dalak, karaciğer, kemik iliği ve lenf düğümünün birlikte değerlendirilmesi ve klinik bulgularla immünohistokimyasal-histopatolojik bulguların birbiriyle ilişkilendirilmesi, tanıyı koymaya çok yardımcı olur ve son tanıya ulaşmamızı sağlar.

Anahtar Kelimeler: Lenfoma, non-Hodkin; splenik neoplazmalar; lösemi, B-hücre

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Leukemias and non-Hodgkin lymphomas (NHL) commonly involve the spleen secondarily as a part of a generalized disease or originate primarily in the spleen, then spread to other sites.[1-3] Primary splenic

lymphoma refers to a subset of NHL in which the disease is thought to begin in the spleen or the bulk of disease is concentrated in the spleen with additional involvement of splenic hilar lymph nodes, reflecting the ambiguity of the term "primary splenic lymphoma".[4] One of the reasons for the infrequent occurrence of primary malignant lymphoma of the spleen is because there are no symptoms until the development of disseminated disease. The spleen is involved in 35% of cases with disseminated NHL and is the dominant site at presentation in only 1%.[5,6] Histological and immunohistochemical studies did not reveal any differences between primary malignant lymphoma of the spleen and disseminated malignant lymphomas with splenic involvement with regard to morphologic features, immunophenotype, host cell infiltrates, or proliferation activity.[4]

Several B-cell lymphomas and leukemic chronic lymphoproliferative disorders, including prolymphocytic leukemia (PLL), hairy cell leukemia (HCL), splenic marginal zone lymphoma (SMZL), lymphoplasmacytic lymphoma (LPL), Waldenstrom's macroglobunemia, B-chronic lymphocytic leukemia (B-CLL), mantle cell lymphoma (MCL), follicular lymphoma (FL), and diffuse large B-cell lymphoma (DLBCL) may manifest with only splenomegaly at presentation.[7-12] The differential diagnosis of splenic lymphomas may be difficult, especially in cases with prominent splenomegaly only, without additional diagnostic yield of the lymph node or bone marrow biopsies.[3] There are only a few studies in the literature, examining the spleen as a diagnostic specimen and fewer which have compared the findings in different subtypes of splenic lymphomas.[3,13,14]

In this retrospective study, we examined the morphologic and immunohistochemical characteristics of 32 cases of primary or secondary splenic B-cell leukemias/lymphomas presenting with splenomegaly in the absence of lymphadenopathy, in which a definitive diagnosis and subclassification was established according to the recent World Health Organization (WHO) classification.[15]

MATERIALS AND METHODS

PATIENTS AND TISSUES

All tissue sections and corresponding pathology reports of patients who underwent splenectomy between January 1990 and December 2004 and were diagnosed with splenic involvement of leukemia/lymphoma were recruited. Cases with unavailable or inadequate diagnostic material for review were not included. After the review, 12 cases, 4 with a diagnosis of T-cell lymphoma and 8 with Hodgkin lymphoma, were excluded from the study. The remaining 32 cases diagnosed with primary or secondary splenic B-cell leukemia/lymphoma consecutively were the focus of the study.

Multiple slides of splenic tissue were evaluated for the presence and pattern of the red and/or white pulp infiltration, morphologic characteristics of the neoplastic cell population and accompanying benign cells, presence or absence of hemorrhage, pseudosinuses, infarcts, "marginal zone" pattern, follicular colonization, residual germinal centers, extracellular hyaline deposits, sclerosis, and necrosis.

In each case, the significant macroscopic findings such as splenic weight, size, and gross appearance of infiltrates were recorded from the original files. Hematoxylin-eosin (H&E) slides were examined blinded, without any knowledge of initial diagnosis and clinical information. Bone marrow, lymph nodes, liver, and/or biopsies from other tissues were analyzed independently, when available.

IMMUNOHISTOCHEMICAL STAINING

The representative formalin-fixed, paraffin-embedded tissue blocks were selected, recut, and 5-μm thick serial sections were subjected to an immunohistochemical study with a large panel of monoclonal antibodies which were listed on Table 1. Paraffin sections were deparaffinized according to standard procedures, rehydrated, and the endogenous peroxidase activity was blocked by incubation in 3% hydrogen peroxide/methanol for 5 minutes. For antigen retrieval, the sections were heated in a pressure cooker in 0.1 mM EDTA (pH 8.0) solution for 2.5 minutes. The sections were

TABLE 1: List of antibodies used in the immunohistochemical study.

Antibody	Clone	Species	Company	Code	Dilution
ALK-1	ALK1	Mouse	DAKO	M7195	1:25
BCL-2	Bcl-2/100/DS	Mouse	Novocastra	NCL-L-Bcl2	1:60
BCL-6	PG-B6p	Mouse	DAKO	M7211	1:10
CD3	PS1	Mouse	Novocastra	NCL-L-CD3-PS1	1:150
CD5	4C7	Mouse	NeoMarkers	MS-393-S	1:40
CD10	56C6	Mouse	Novocastra	NCL-L-CD10-270	1:100
CD20	L26	Mouse	Novocastra	NCL-L-CD20-L.26	1:150
CD21	2G9	Mouse	Novocastra	NCL-CD21-2G9	1:10
CD23	1B12	Mouse	Novocastra	NCL-L-CD23-1B12	1:30
CD30	Ber-H2	Mouse	DAKO	M0751	1:30
CD43	DF-T1	Mouse	DAKO	M0786	1:40
Cyclin D1	SP4	Rabbit	NeoMarkers	RM-9104-S	1:100
Ki-67	MIB-1	Mouse	DAKO	M7248	1:150
TRAP	26E5	Mouse	Novocastra	NCL-TRAP	1:50

then treated with blocking solution, followed by incubation with monoclonal primary antibodies for 30 minutes at room temperature. Immunohistochemical staining was performed by Dako EnVisionTM kit (Dako, Denmark). Diaminobenzidine (DAB, Dako, Denmark) in the presence of hydrogen peroxide was used as the chromogen and Gill's hematoxylin for counterstaining. Positive control tissue sections were run in parallel with each batch. The lymphoid markers had internal controls stained on all sections, except for ALK1 and TRAP. Primary antibodies were omitted in negative controls and were replaced by nonimmune serum.

SCORING AND STATISTICAL ANALYSIS

The evaluation of immunohistochemical staining was performed by two pathologists (F.K.D. and M.H.). High magnification fields of tumors were chosen for evaluation of Ki-67, counting up to 500 cells, excluding small T-cells and other nonneoplastic cells. All cases included in the study were classified according to the currently accepted criteria of the WHO classification.[15] The results and relevant information, obtained at the time of initial diagnosis, were compared with those at the final diagnoses.

The SPSS software package for Windows (version 11.5, SPSS, Chicago, IL) was used for all statistical analyses. The values of mean ± SD were analyzed. The Kruskal-Wallis test was performed to compare the Ki-67 index in the groups of the final diagnosis. One case diagnosed with T-cell-rich B-cell lymphoma (TCRBCL) was included in the DLBCL group, for the purpose of statistical analysis. Kappa (κ) analysis was used to measure the correlation between the initial and final diagnoses.

RESULTS

PATIENT CHARACTERISTICS

Thirty-two cases of splenic B-cell leukemia/lymphoma were eligible for evaluation and characteristics of the patients were presented on Table 2. There were 19 (59.4%) female and 13 (40.6%) male patients with a male-to-female ratio of 1.5:1 aged 32-80 years (median, 58 years). After evaluation of the morphological and immunohistochemical features of the cases, the final diagnoses reached were as follows: 11 (34.4%) HCL, 8 (25%) SMZL, 8 (25%) DLBCL including 1 TCRBCL, 4 (12.5%) MCL, and 1 (3.1%) PLL. Twenty cases of B-cell lymphoma (SMZL, DLBCL, TCRBCL, and MCL) were all cases presenting with splenic involvement, without any other nodal or extranodal disease involvement by computed tomography (CT) scan or physical examination at the time of diagnosis. Former cases mostly diagnosed as HCL and one chal-

TABLE 2: Age, sex, splenic weight, and involvement of accessory spleen, lymph nodes, bone marrow and/or other extranodal sites in patients of splenic B-cell leukemia/lymphoma.

Diagnoses	HCL (n= 11)	SMZL (n= 8)	DLBCL (n= 8)	MCL (n= 4)	PLL (n= 1)	Total (n= 32)
Age mean ± SD (years)	58.91 ± 13.49	57.40 ± 15.14	57.00 ± 7.66	57.75 ± 12.31	63.00	58.19 ± 11.64
Sex (male:female)	6:5	1:7	4:4	2:2	0:1	13:19
Splenic weight mean ± SD (g)	1262.90 ± 761.24	1761.86 ± 1140.49	1528.60 ± 913.77	1700 ± 848.52	957	1 1494.25 ± 892.96
Accessory spleen (+/-/NA)	0/1/10	2/1/5	2/0/6	0/0/4	0/1/0	4/3/25
Splenic hilar LN (+/-/NA)	1/1/9	4/2/2	3/2/3	3/1/0	0/1/0	11/7/14
Distant LN (+/-/NA)	0/0/11	0/0/8	1/0/7	0/0/4	0/1/0	1/1/30
Bone marrow (+/-/NA)	7/1/3	2/1/5	4/1/3	2/0/2	1/0/0	16/3/13
Other organs (+/-/NA)	1/2/8	0/0/8	3/2/3	2/0/2	0/0/1	6/4/22

Abbr. HCL: hairy cell leukemia, SMZL: splenic marginal zone lymphoma, DLBCL: diffuse large B-cell lymphoma, MCL: mantle cell lymphoma, PLL: prolymphocytic leukemia, LN: lymph nodes, NA: material not available for histopathologic examination.

lenging case of PLL were also included to discuss the differential diagnostic features of leukemic involvement of spleen. Splenectomy was necessary for diagnostic purposes in all cases of lymphoma, while cases presenting with leukemia had undergone splenectomy mostly for therapeutic/palliative purposes.

GROSS TUMOR CHARACTERISTICS

Paraffin-embedded tissue blocks were sent for consultation in 8 cases without any description of the gross features, so macroscopic findings were reported for 24 cases. The splenic weight ranged from 261 to 4230 g (mean 1494 ± 892.96). For each subtype of leukemia/lymphoma, the weight ranges were as follows: HCL, 261 to 3000 g; SMZL, 1030 to 4230 g; DLBCL, 637 to 2850 g; MCL, 1100 to 2300 g; and the PLL case 957 g. Gross examination revealed a predominant diffuse infiltration of the red pulp in 12 cases (HCL, 11/11; DLBCL 1/8). The remaining cases with a predominant white pulp infiltration presented a macronodular pattern in 3 cases (DLBCL, 3/8), micronodular pattern in 13 cases (SMZL, 7/8; MCL, 4/4; TCRBCL, 1/1; PLL, 1/1), and a combined macro- and micronodular pattern in 4 cases (DLBCL, 3/8; SMZL, 1/8).

MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL FINDINGS OF VARIOUS HISTOPATHOLOGICAL TYPES

Hairy cell leukemia

Eleven of 32 cases were diagnosed with HCL, involving predominantly the red pulp of the spleen. The red pulp infiltration was diffuse in all except one with diffuse and nodular involvement (Figure 1A). Hemorrhage and pseudosinuses (blood lakes surrounded by hairy cells) were identified in 9 cases (Figure 1B). Neoplastic cells were small and monomorphic (Figure 1C). Two cases presented with infarcts. Marginal zone differentiation or follicular colonization were absent in all, extracellular hyaline was seen in one case, and sclerosis in another one. Residual germinal centers were present in only two cases.

The neoplastic cells in all cases were CD20 and TRAP-positive, and all cases were negative for CD3, CD5, CD23, CD30, and CD43 (Table 3). The neoplastic cells were CD10-positive in two cases and CD21-positive in one. Cyclin D1 demonstra-

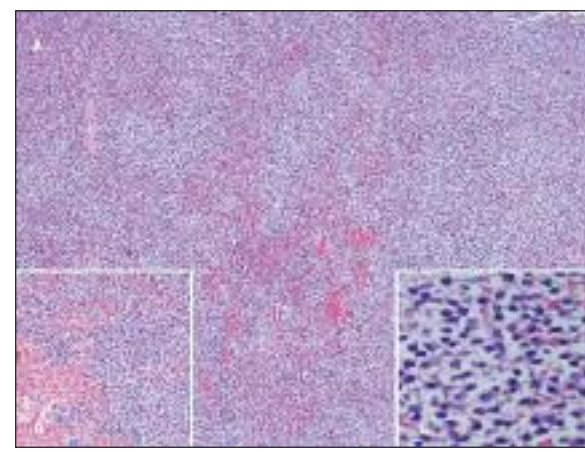

FIGURE 1: Hairy cell leukemia infiltrating the spleen. **A**, Diffuse infiltration of the red pulp (H&E, x4). **B**, Blood lakes lined by neoplastic cells (H&E, x10). **C**, Small neoplastic cells with clear cytoplasms (H&E, x40).

TABLE 3: The results of the immunohistochemical staining.

Case	Diagnosis	CD20	CD3	CD5	CD10	CD21	CD23	CD43	cylin D1	TRAP	bcl-2	bcl-6	CD30	%Ki-67
1	HCL	+	-	-	-	-	-	-	-	+	+	-	-	1
2	HCL	+	-	-	-	+	-	-	-	+	+	-	-	3
3	HCL	+	-	-	+	-	-	-	-	+	+	-	-	3
4	HCL	+	-	-	-	-	-	-	-	+	+	-	-	2
5	HCL	+	-	-	-	-	-	-	+	+	+	-	-	1
6	HCL	+	-	-	-	-	-	-	-	+	+	-	-	0
7	HCL	+	-	-	+	-	-	-	-	+	+	-	-	1
8	HCL	+	-	-	-	-	-	-	-	+	-	-	-	3
9	HCL	+	-	-	-	-	-	-	-	+	+	-	-	1
10	HCL	+	-	-	-	-	-	-	-	+	+	-	-	2
11	HCL	+	-	-	-	-	-	-	-	+	-	-	-	1
12	SMZL	+	-	-	-	-	-	-	-	-	+	+	-	2
13	SMZL	+	-	-	-	-	+	-	-	+	+	+	-	5
14	SMZL	+	-	-	-	-	-	-	-	+	+	-	-	5
15	SMZL	+	-	-	-	-	-	-	-	-	+	-	-	4
16	SMZL	+	-	-	-	-	-	-	-	-	+	-	-	15
17	SMZL	+	-	-	-	-	-	-	-	-	+	+	-	1
18	SMZL	+	-	-	-	-	-	-	-	-	+	+	-	0
19	SMZL	+	-	-	-	-	-	-	-	-	+	-	-	7
20	DLBCL	+	-	-	-	-	-	-	-	-	+	-	+	40
21	DLBCL	+	-	-	-	-	-	-	-	-	-	+	-	40
22	DLBCL	+	-	-	-	-	-	-	-	-	+	+	+	1
23	DLBCL	+	-	-	-	-	-	-	-	-	+	+	+	65
24	DLBCL	+	-	-	-	-	-	-	-	-	+	-	+	1
25	DLBCL	+	-	-	-	-	-	-	-	-	-	-	-	0
26	DLBCL	+	-	-	-	-	-	-	-	-	-	-	-	52
27	TCRBCL	+	-	-	-	+	-	-	-	-	-	-	-	1
28	MCL	+	-	+	-	-	-	+	+	+	+	-	-	8
29	MCL	+	-	+	-	-	-	-	+	-	+	-	-	12
30	MCL	+	-	+	-	-	-	+	+	+	+	-	-	10
31	MCL	+	-	-	-	-	-	+	+	-	+	-	-	20
32	PLL	+	-	+	-	-	-	+	-	+	+	-	-	5

Abbr. HCL: hairy cell leukemia, SMZL: splenic marginal zone lymphoma, DLBCL: diffuse large B-cell lymphoma, MCL: mantle cell lymphoma, PLL: prolymphocytic leukemia.

ted a weak and focal nuclear reactivity in one case. Bcl-2 was positive in all except two cases. Ki-67 proliferation index ranged between 0 and 3 (mean 1.67 ± 1.12).

Accessory spleen was present in one case, but without involvement. Splenic hilar lymph nodes were available in two cases and one was involved. Bone marrow biopsy was available in 8 cases and 7 had neoplastic infiltrate. Three cases had undergone liver biopsies and 1 had hepatic involvement.

Splenic marginal zone lymphoma

Eight cases were classified as SMZL, all but one presenting with a predominant white pulp expansion (micronodular infiltration) and one with accompanying macronodular infiltration (Figure 2A). On microscopic evaluation, marginal zone differentiation was observed in all except the latter (Figure 2B and 2C). Residual germinal centers were present within the nodular infiltrations in 4 cases and colonization of follicles was seen in 3. Accom-

panying red pulp infiltration was identified in all cases forming numerous small nodules and cords within sinusoids, and in one case, the red pulp involvement was predominant with a mixed diffuse and nodular pattern. Extracellular hyaline deposits were present in all but one, usually within the centers of micronodules. Occasional epithelioid histiocytes were prominent in 4 cases, in the absence of a distinct granuloma formation. Only 1 case had a large number of plasma cells. Sclerosis was present in 2 cases. There was hemorrhage in 3 cases, with pseudosinuses in 1 of those. Proliferation centers, infarcts and necrosis were absent.

In all cases, the neoplastic cells were CD20 and bcl-2-positive and negative for CD3, CD5, CD10, CD21, CD30, CD43, and cyclin D1 (Table 3). Bcl-6 was positive in half of the cases. Two cases were positive with TRAP, and 1 of those was CD23-positive. Immunostaining of residual germinal centers with CD21 revealed that germinal centers were preserved in all cases, although not apparent on H&E stained sections. Ki-67 proliferation index range was 0-15 (mean 6.40 ± 5.46).

Three cases had accessory spleens removed and 2 harbored neoplastic infiltrates. Splenic hilar lymph nodes were available in 6 cases and 4 were involved. Bone marrow biopsies were available in 3 cases and 2 were infiltrated. One of these cases presented circulating villous lymphocytes in peripheral blood.

Diffuse large B-cell lymphoma

Seven cases were classified as DLBCL and one as micronodular TCRBCL. The main tumor mass was in the white pulp in 7 cases, including the TCRBCL case. The white pulp infiltration presented with a macronodular pattern in 3 cases, macro- and micronodular pattern in another three, and only micronodular in one, the TCRBCL (Figure 3A and 3B). A nodular neoplastic infiltrate was seen in the red pulp in all, and in one case, the red pulp infiltration was predominant, diffuse and nodular in pattern. Neoplastic cells were polymorphic in all cases, ranging from small to large cells in three and were composed of only large cells in the remaining. There was hemorrhage in 5 cases and 3 of those had splenic infarcts. There were no pseudosinuses, marginal zone differentiation, follicular colonization, residual germinal centers, and extracellular hyaline. Sclerosis was present in 6 cases and was severe in 2.

In all cases the neoplastic cells were CD20-positive and CD3, CD5, CD10, CD23, CD43, cyclin D1, and TRAP-negative (Figure 3C, 3D, Table 3). There was one TCRBCL case positive for CD21 and negative for CD30, bcl-2 and bcl-6. Four cases coexpressed CD30 and bcl-2. Bcl-6 was positive in 3 cases. Two cases coexpressed CD30, bcl-2 and bcl-6. Ki-67 proliferation index ranged between 0 and 65 (mean 29.40 ± 27.86).

Accessory spleen existed in 2 cases; both were infiltrated. Four cases had hilar lymph nodes removed and 2 were infiltrated. Bone marrow biopsy was available in 4 cases and 3 had neoplastic infiltrates. Three of 5 cases with liver biopsies performed had hepatic involvement. One case had involvement of the colon resected with the spleen. The case with TCRBCL presented hilar lymph node, bone marrow, and a peripheral lymph node involvement, both of the latter biopsied after splenectomy for staging purposes.

Mantle cell lymphoma

Four cases of MCL involving the spleen, all characterized by an expansion of the white pulp, were studied. Minimal infiltration of the red pulp was identified in the form of small nodules. The neoplastic infiltration was composed of monomorphic small lymphocytes with scant cytoplasm. The nuclei of the cells showed a coarse chromatin pattern and irregular nuclear contours. Nucleoli were not distinct. Hemorrhage, infarcts, and necrosis were present in one case. Two cases presented with marginal zone differentiation, one with follicular colonization, a few small residual germinal centers, and extracellular hyaline.

In all cases the neoplastic cells were positive with CD20, cyclin D1, and bcl-2 but negative for CD3, CD10, CD21, CD23, bcl-6, and CD30 (Table 3). No staining of neoplastic cells was observed with CD5 in 1 case and CD43 in another one. Interestingly, TRAP was positive in the 2 cases showing

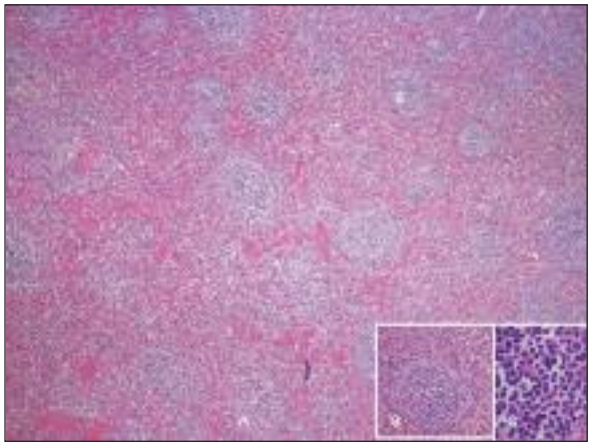

FIGURE 2: Splenic marginal zone lymphoma. **A,** A predominant white pulp expansion with accompanying red pulp infiltration forming numerous small nodules (H&E, x4). **B**, Marginal zone differentiation (H&E, x10). **C**, Neoplastic cells expanding the marginal zone and infiltrating the red pulp (H&E, x40).

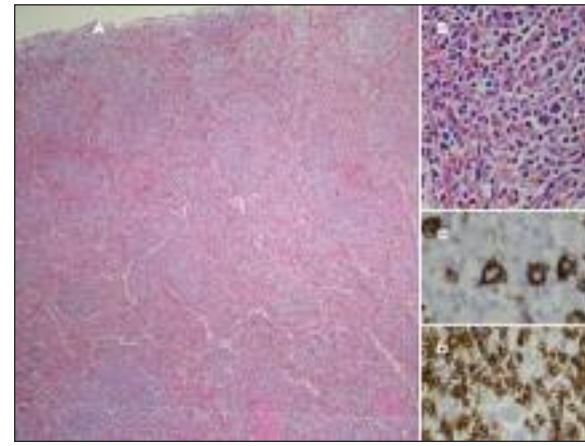

FIGURE 3: T-cell-rich B-cell lymphoma infiltrating the spleen. **A**, The micronodular pattern of white pulp infiltration (H&E, x4). **B**, Scattered large neoplastic cells surrounded by small reactive lymphocytes (H&E, x40). **C**, CD20 staining of neoplastic B-cells (immunoperoxidase, DAB, x40). **D**, CD3-positive reactive small T-cells surrounding the neoplastic large cells (immunoperoxidase, DAB, x40).

coexpression of CD5 and CD43. Ki-67 proliferation index range was 8-20 (mean 12.67 ± 6.43).

Accessory spleens did not exist in any 1 of the specimens, but hilar lymph nodes were present in all 4, with involvement in 3. Bone marrow and liver biopsies were available in 2 of these latter cases and were both involved. In 1 of these latter cases, the diagnosis was verified by endoscopic biopsy of the rectum.

Prolymphocytic leukemia

Only 1 case of PLL with expansion of the white pulp was included in the study (Figure 4A). A nodular and diffuse infiltration of the red pulp was also present. The neoplastic cells were polymorphic, primarily large cells with round, vesicular nuclei and centrally located distinct nucleoli (prolymphocytes) admixed with small mature lymphocytes (Figure 4B). This case was the only one demonstrating hemophagocytosis in neoplastic cells. There was minimal hemorrhage, but no proliferation centers, pseudosinuses, infarcts, "marginal zone" pattern, follicular colonization, residual germinal centers, extracellular hyaline deposits, sclerosis, and necrosis.

CD20, CD5, CD43, and bcl-2 were positive on immunohistochemical staining (Table 3); CD23 was absent. Other lymphoid markers included in the study (CD3, CD10, CD21, cyclin D1, bcl-6, CD30) were negative in neoplastic cells, except for TRAP (Figure 4C). Ki-67 proliferation index was low (5%).

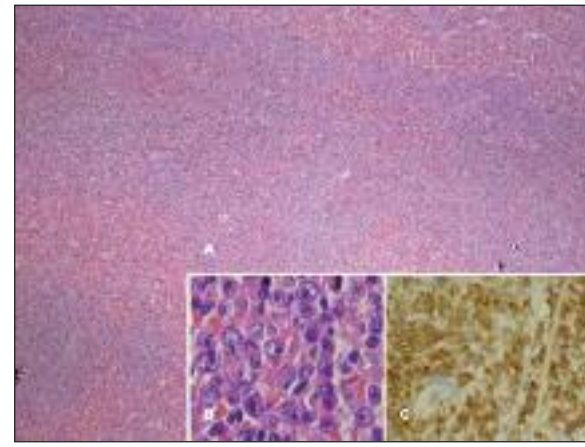

FIGURE 4: B-prolymphocytic leukemia infiltrating the spleen. **A**, Diffuse infiltration of the white pulp (H&E, x4). **B**, Prolymphocytes with round, vesicular nuclei and centrally located distinct nucleoli (H&E, x100). **C**, TRAP positivity of neoplastic cells (immunoperoxidase, DAB, x40).

This case presented with a high lymphocyte count, anemia, thrombocytopenia, and splenomegaly and was diagnosed with B-cell prolymphocytic leukemia. Because some patients with B-cell prolymphocytic leukemia presenting with massive splenomegaly may be effectively palliated with splenectomy, this case, on therapy with

fludarabine for over a year, had undergone splenectomy.

DISCUSSION

In the current study, we applied a panel of monoclonal antibodies reactive with immunohistochemical techniques on formalin-fixed, paraffin-embedded tissue sections and overviewed the differential diagnostic features of splenic B-cell lymphoma and leukemia. Twenty patients with B-cell lymphoma, who had undergone splenectomy for splenomegaly or splenic mass lesions, had no evidence of lymphadenopathy or other extranodal disease by clinical examination at presentation. Since splenic lymphomas present a wide variety of morphologies, previous studies have produced conflicting results, such as some introducing low-grade lymphomas, not otherwise specified or MCL, whereas others favoring small lymphocytic lymphoma, SMZL or DLBCL as the most common histopathological subtypes.[6,16-19] In this series, the most common type of splenic involvement by B-cell lymphoma was SMZL, followed by DLBCL. Differential diagnosis may be difficult in some cases and a detailed immunohistochemical study is a *sine qua non* for a definite diagnosis according to the WHO criteria.[15]

The recent description of SMZL, a distinct B-cell lymphoma with characteristic clinical, histological, and immunologic features, have awakened wide interest in splenic lymphomas.[8,20-23] SMZL is a morphologically well-defined entity composed of predominantly small to medium sized lymphocytes, characterized by marginal zone differentiation, follicular colonization, plasmacytic differentiation, and a distinctive immunophenotype: $CD20^+$, $bcl\text{-}2^+$, $CD43^-$, $CD5^-$, $CD10^-$, $CD23^-$, $bcl\text{-}6^-$, and cyclin $D1^-$ (3, 5, 9, 10, 15, 20-23). In this study, marginal zone differentiation and extracellular hyaline was noted in the majority of cases (7/8, 87.50%) while plasmacytic differentiation was identified in only 1 case. Thus, we believe that marginal zone differentiation and extracellular hyaline are the two most important histopathological criteria for the diagnosis of SMZL. However, TRAP was positive in 2 cases of SMZL in the present study, questioning the reliability of this marker for the diagnosis of HCL. Among low-grade lymphoid neoplasms various disorders are considered in the differential diagnosis of SMZL.[21] Most involve the white pulp in a similar pattern on low-power examination, except HCL infiltrating the red pulp.[3,20] The most difficult scenario is distinguishing SMZL from follicular lymphoma with marginal zone differentiation.[9] The difference in bcl-2 staining patterns, mainly the homogeneous staining of follicles in follicular lymphoma and the colonization pattern of SMZL, was reported to be helpful in this differential diagnosis, and this feature was identified in 3 SMZL cases, presenting with widespread follicular colonization.[20]

All the cases of HCL in the present study had characteristic morphological appearance in the spleen and bone marrow biopsies. The involvement of the red pulp was in contrast to the white pulp infiltration displayed by other low-grade B-cell lymphomas and was the most useful histopathological finding on low-power evaluation. In addition, blood lakes, a very distinguishing feature of this tumor, were present in all HCL cases. Immunohistochemical staining of TRAP, a classical, simple, sensitive and quite specific method for detection of HCL cells, was expressed in all cases, but one presented very weak reactivity.[24] As mentioned above, TRAP positivity was identified in 2 cases of SMZL, 2 cases of mantle cell lymphoma, and 1 case of B-cell PLL. Although immunohistochemical staining of TRAP was suggested to be an extremely sensitive marker of HCL with a specificity of 98.27%, TRAP positivity has been reported in several lymphoid malignancies, including B-cell CLL, B-cell PLL, T-cell CLL, and SMZL.[12,21,24,25] Thus, TRAP staining does not always lead to a direct diagnosis of HCL and one should be very cautious when interpreting a positive staining with TRAP, if a diffuse red pulp infiltration and blood lakes are not identified in a given case. Otherwise, typical HCL cases might manifest with immunophenotypic aberrations from the characteristic pattern; in the previously published reports, CD10 expression was the most common variation, presented in 25% of cases in one large series, also noted in 2 cases of the

present series.[12,26,27] Flow cytometric expression of CD103 might be the sole identifying feature on differential work-up of the cases with aberrant features.

There are a few series of splenic DLBCL in the literature, reporting that low-grade lymphoproliferative disorders may rarely undergo large cell transformation.[10,11,28] However, none of the 8 cases of DLBCL in the present study had a clinical history of progression from a low-grade disease. There was 1 case with diffuse splenic involvement, which apparently originated from the red pulp, rather than the white pulp. There are some reports of DLBCL that primarily involve the splenic red pulp in a diffuse pattern.[28-31] In those cases, the differential diagnosis involves grade 3 follicular lymphoma, which could be excluded by the lack of follicular pattern, absence of follicular dendritic cells on CD21 staining, and lack of CD10 expression. Some rare cases are reported to have a micronodular pattern with a TCRBCL composition and 1 of the cases in the present series represented this entity.[11,32,33] As well known, TCRBCL is a unique morphologic variant of DLBCL that consists of a small proportion of large neoplastic B-cells within a prominent component of reactive T-cells and/or histiocytes.[31-34]

Data from the literature concerning the histology of MCL in the spleen are very few and overlapping features exist to a certain extent.[6,7] MCL cases have a predominantly white pulp involvement like other small B-cell-lymphomas. Immunohistochemistry is very helpful in reaching the definite diagnosis of MCL. In this study, all cases of MCL coexpressed cyclin D1 and CD5, except for one cyclin $D1^+$, $CD5^-$ case. The absence of CD5 expression has been reported in about 20% of MCLs.[6]

It has recently been suggested that B-PLL harboring t(11;14)(q13;q32) presents with younger age, male predominance, and extranodal involvement compared to those patients without t(11;14) with an infiltration of cells resembling prolymphocytes or cells of MCL, and thus may represent a splenomegalic form of MCL evolving with leukaemia.[34] The only 1 case of B-PLL in this series was $CD20^+$, $CD5^+$, $CD43^+$, $TRAP^+$, bcl-2^+, $CD23^-$, $CD10^-$, $CD21^-$, and cyclin $D1^-$. This case had features resembling those of the blastoid form of MCL, but the examination of the infiltration in the bone marrow biopsy and the low Ki-67 score, together with cyclin D1 negativity revealed the diagnosis of B-PLL. Because the circulating cells may resemble prolymphocytes in hairy cell variant, the likelihood of hairy cell variant, which usually lacks the typical hairy cell antigens such as CD25, CD103 and TRAP, was also considered in the differential diagnosis of this challenging case. However, the examination of the bone marrow aspirate and biopsy revealed features typical of PLL and the splenic white pulp predominant infiltration consisting mainly of cells with features of prolymphocytes confirmed the diagnosis.

Generally, widespread dissemination with involvement of the hilar lymph node, liver and bone marrow is a frequently encountered feature of B-cell lymphoproliferative diseases of the spleen. In the current study, 17 cases had hilar lymph nodes removed and 11 had neoplastic infiltrates (65%). Sometimes histopathological evaluation of hilar lymph node is very useful in reaching a final diagnosis. Therefore, hilar lymph nodes should always be looked for on gross handling of splenectomy specimens. Eighteen cases had bone marrow biopsies performed and 15 had neoplastic infiltrates (83%). Six cases had accessory spleen removed and 4 had neoplastic infiltrates (67%). Twelve cases had liver biopsies performed and 6 had neoplastic infiltrates (50%).

In this study, no significant difference was observed in the Ki-67 index between the groups investigated (x^2=8.602, p= 0.072). The correlation between the initial and final diagnoses were strong for the cases of HCL (K=1.000, p=0.000), SMZL (K=0.833, p=0.00), DLBCL (K=0.818, p=0.00), and PLL (K=1.000, p=0.000), but not for MCL (K=0.368, p=0.07).

This study revealed that splenic B-cell lymphoproliferative diseases were a heterogeneous group of B-cell leukemia/lymphomas with various overlapping features, which often led to difficulties in differential diagnosis. In the prob-

lematic cases with massive involvement of the spleen, but no evidence of lymphadenopathy by CT scan or physical examination, a detailed overall assessment and correlation of the histopathological and immunohistochemical features of the spleen, accessory spleen, hilar lymph nodes, bone marrow and liver biopsies are most helpful and usually lead to final diagnosis in most cases.

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