ORIGINAL ARTICLE

# Histopathological audit of splenectomies received at a cancer hospital

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## ABSTRACT

**Background:** There are few studies in the literature studying the yield of the diagnostic splenectomy in a suspicious lymphoma case. Moreover, their relevance is limited owing to low number of cases, the use of selection criteria, and the lack of modern ancillary studies. We present a histopathological review of splenectomy specimens referred as a case of lymphoma to our center. **Materials and Methods:** The medical charts and laboratory data on all patients of all splenectomy specimens between the years 2003 and 2008 were reviewed. Morphological and immunohistochemical features were analyzed and the lymphomas were sub-typed in accordance to 2008 WHO Classification of Hematolymphoid Neoplasms. Flow cytometry immunophenotyping available in few cases was correlated. **Results:** A total of 46 cases studied included splenic marginal zone lymphoma (SMZL) (19 cases), splenic diffuse large B-cell lymphoma (DLBCL) (14 cases), splenic diffuse red pulp B-cell lymphoma (DRP) (five cases), follicular lymphoma (three cases), hairy cell leukemia (HCL) (two cases), HCL variant (HCLv) (1 case), 1 case of hepatosplenic gamma delta T-cell lymphoma (TCL), and 1 cases of TCL (not otherwise specified). **Conclusions:** Predominantly splenic lymphoma is a biologically heterogeneous entity, ranging from low-grade SMZL to high-grade DLBCLs. TCLs constituted only 4% of all our cases. DRP, HCL, and HCLv have similar diffuse red pulp patterns of splenic involvement and are differentiated based on flow cytometric immunophenotyping. We had a large number of splenic DLBCL and none of these involved bone marrow (BM), while all other lymphoma subtypes had BM involvement (stage IV disease). Morphological and immunophenotypic (immunohistochemistry and flow cytometry) features of BM and splenectomy specimen need to be correlated to differentiate these rare though similar-looking entities with overlapping features.

**KEY WORDS:** Primary splenic lymphoma, predominantly splenic lymphoma, splenectomy, splenic marginal zone lymphoma, splenic diffuse red pulp B-cell lymphoma, splenic diffuse large B-cell lymphoma

## INTRODUCTION

Splenic involvement in lymphomas is common; however, primary splenic lymphoma (PSL) is a poorly defined entity and constitutes approximately 1% of all lymphomas.[1,2] Splenic lymphoma is a generalized disease with predominant splenic component as bone marrow (BM) is involved in most of these cases.[1-3] Thus, most of these cases are predominantly splenic lymphomas (PdSL). Most cases of splenic lymphomas are diagnosed based on a peripheral blood smear examination substantiated with flow cytometric (FCM) immunophenotyping and do not require splenectomy. WHO classification of hematolymphoid neoplasms does not mention about these entities, although has broadly sub-classified splenic lymphomas into splenic B-cell marginal zone lymphoma (SMZL) and splenic B-cell lymphoma/leukemia, unclassifiable which further include splenic diffuse red pulp small B-cell lymphoma (DRP) and hairy cell leukemia variant (HCLv).[3] Neoplasms like hairy cell leukemia (HCL) and HCLv are primarily diagnosed based on FCM immunophenotyping of blood/BM and splenectomy is generally not required for diagnosis. Subtypes like SMZL and Diffuse large B-cell lymphoma (DLBCL) might require splenectomy for diagnosis in the absence of marrow and nodal involvement.[4] DRP is a diagnosis based on splenectomy specimen.

Splenectomy may be performed for diagnostic as well as therapeutic purposes. There are few studies in the literature studying the yield of the diagnostic splenectomy in a suspicious lymphoma case.[5] We present PdSL diagnosed on splenectomy specimens and morphologically and immunohistochemically subtype them in accordance with WHO 2008 classification.[4]

## MATERIALS AND METHODS

We analyzed 46 consecutive splenectomy specimens/paraffin blocks referred as

lymphoma, over a six-year period (between 2003 and 2008). Twenty seven of these were the referrals with sketchy clinical details, although adequate follow-up was available in the 19 cases only. Diagnosis in the splenectomy specimen was based primarily on morphology which was further substantiated by immunohistochemistry (IHC). Additional material was correlated in available cases, BM Aspirate (18 cases), BM Biopsy (16 cases), and FCM immunophenotyping (9 cases). A battery of immunomarkers including LCA, CD20, CD3, CD5, CD10, CD23, FMC7, CD43, IgG, IgD, Light chain restriction, CD79a, Bcl-2, and cyclin D1 immunostaining was employed to help in narrowing down the diagnosis. IgM and IgD could be performed on a few cases only, both by FCM as well as by IHC. Those cases of splenic lymphomas were not included where splenectomy specimen/ paraffin blocks were not available.

## RESULTS

These 46 cases of PdSL included 19 cases of SMZL, 14 cases of DLBCL, 5 cases of DRP, 3 cases of follicular lymphoma (FL), 2 cases of HCL, 1 case of HCL variant, 1 case of T-cell lymphoma - not otherwise specified (TCL-NOS), and 1 case of hepatosplenic gamma delta T-cell lymphoma (HSGDTCL) [Table 1].

### Splenic Marginal Zone Lymphoma

There were 10 male and 9 female patients; median age was 53 years (range, 41-83 years). Clinical details were available in seven cases only. Left hypochondriac pain and abdominal discomfort or pain was common presenting symptoms. In addition, one patient presented with breathlessness and another with recurrent bouts of vomiting. Anemia was seen in six cases, with two patients having hemoglobin less than 9 gm. Five patients had leukocytosis, maximum 49 000/cmm and four of them had lymphocytosis (range, 51-86%). One patient had thrombocytopenia. Complete blood counts (CBC) were normal in one case. Serum globulins were raised in six cases. Serum electrophoresis was not done. Serum alkaline phosphatase was marginally raised in three cases. Serology for *hepatitis c virus* (HCV), or *human immune deficiency virus* (HIV), or *hepatitis B virus* (HBV) was negative in all 19 cases of splenic lymphomas, including seven cases of SMZL.

BM aspirate was available in six cases and showed involvement in five cases. Aspirate revealed lymphocytosis in four cases (range, 42-79%). One case revealed only 12% lymphocytes (mature looking) on BM aspirate which on immunophenotyping revealed a clonal B-cell population suggestive of involvement by SMZL. FCM immunophenotyping was available in four cases. Tumor cells expressed CD19, CD20, FMC7, CD79a, CD22, and IgM. Light chain restriction was seen in all cases. Weak IgD expression was seen in two cases. CD11c was negative in all three cases. BM biopsy was involved in all six cases. Nodular intertrabecular lymphoid aggregates were the prominent finding, seen in four cases (along with interstitial pattern in three and intrasinusoidal pattern in one). Paratrabecular infiltrates were seen in two cases, one in addition showed interstitial pattern (picked up by CD20 staining).

Splenic size varied from 18 to 37 cm (seven cases). Grossly, the spleens were uniform, red, and congested in all, except one where cut section revealed generalized tiny whitish nodules. Capsule was thickened in all seven cases. Microscopy revealed a predominant micronodular pattern in the white pulp with spillage of tumor cells in the red pulp [Figures 1 and 2]. Four cases showed a mixed pattern, involving both white as well as red

**Table 1: Subtypes of splenic lymphoma and patterns of bone marrow involvement**

| *Subtype* | *Total number of cases n = 46* | *Total BM aspirate n = 18* | *Total BM biopsy n = 16* | *Immunophenotyping by FCM n = 9* | | | |
|---|---|---|---|---|---|---|---|
| | | *Number of cases* | *Involved* | *Number of cases* | *Involved (with pattern)* | *Number of cases* | *Involved* |
| SMZL | 19 | 6 | 5 | 6 | 6<br>5 cases had a predominant nodular pattern admixed with interstitial-3, paratrabecular-1, intrasinusoidal-1<br>1 case had paratrabecular pattern only | 3 | 3 |
| DRP | 5 | 3 | 1 | 2 | 2<br>1 - intrasinusoidal<br>1 - nodular | 2 | 2 |
| HCL | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| HCL-v | 1 | 1 | 1 | 1 | 1<br>interstitial and paratrabecular | 1 | 1 |
| FL | 3 | 2 | 2 | 2 | 2<br>1 - paratrabecular<br>1 - Interstitial | 2 | 2 |
| DLBCL | 14 | 5 | 0 | 5 | 0 | 0 | 0 |
| TCL-NOS | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| HSGDTCL | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

BM - Bone marrow; FCM - Flow cytometric; SMZL - Splenic marginal zone lymphoma; DRP - Diffuse red pulp B-cell lymphoma; HCL - Hairy cell leukemia; HCL-v - HCL variant; FL - Follicular lymphoma; DLBCL - Diffuse large B-cell lymphoma; TCL-NOS - T-cell lymphoma - not otherwise specified; HSGDTCL - Hepatosplenic gamma delta T-cell lymphoma

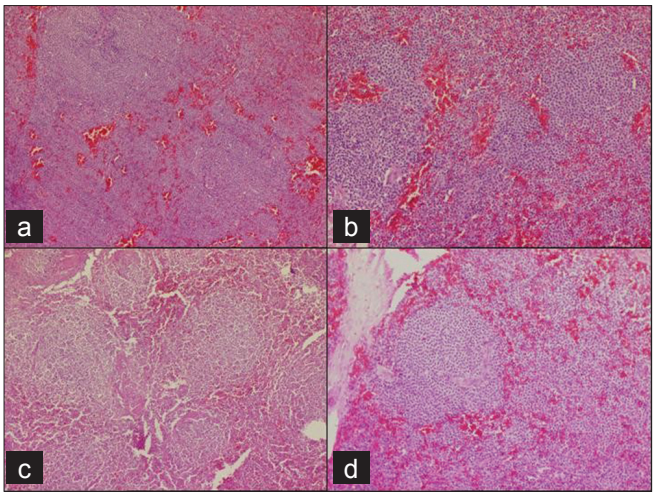


**Figure 1: (a-d) Splenic marginal zone lymphoma showing nodular patterns of tumor cells (H and E, stain, ×40)**

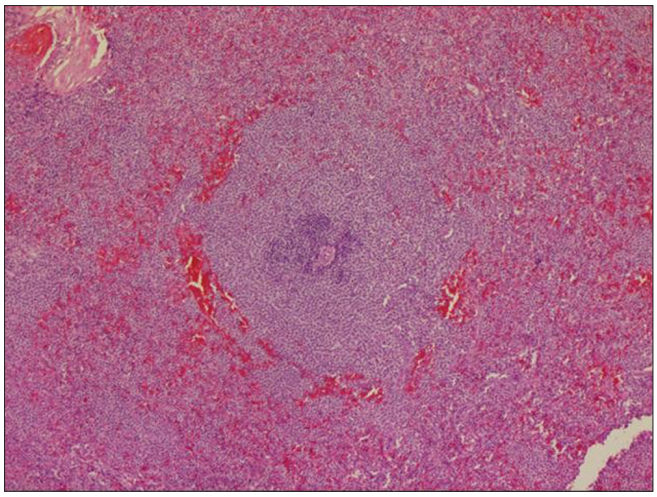

**Figure 2: Splenic marginal zone lymphoma showing nodular patterns of tumor cells (H and E, ×100)**

pulp. One case had a focal nodular pattern in the white pulp and a predominant diffuse pattern in the red pulp. White pulp with residual germinal centers was seen in three cases. Most cases had uniformly sized nodules; however, three cases revealed marked variation in the size of the nodules. Another three cases showed back to back arrangement of nodules. Typical marginal zone expansion was seen in four cases only. There was a prominence of hyalinized vascular channels in six cases. These nodules were rich in small size lymphoid cells with a round nucleus, condensed nuclear chromatin, and moderate amount of clear cytoplasm (monocytoid B cells). There was a sprinkling of large size cells with prominent nucleoli, centroblast like (in varying numbers) in 11 cases. Five cases had a prominence of plasma cells. Six cases (29%) showed a prominence of cleaved lymphoid cells (centrocyte like) admixed with monocytoid B-cells. One case showed predominant cleaved lymphoid cell morphology, where FL and mantle cell lymphoma (MCL) were excluded on the basis of immunophenotyping (CD19+, CD5-. CD10- and cyclin D1-). All 19 cases of SMZL expressed CD20 and Bcl2 was positive in all five cases. Tumor cells expressed IgM and IgD in both cases. CD5 (13 cases), CD23 (11 cases), and CD10 (10 cases) were negative in all cases. Ki67 (Mib1) proliferation index was low, ranged between 5 and 10% in six cases.

Seven cases had lymph node involvement. Four had splenic hilar nodes, one supraclavicular nodes, and another one had generalized abdominal lymphadenopathy (based on CT scan). Liver biopsy was available in two cases and both revealed intrasinusoidal infiltration by small B-lymphoid cells. Cytogenetics was available in three cases. Two had a normal karyotype and one revealed Trisomy 12. Six of these cases are on a regular follow-up (8 months to 4 and half years). None of them received chemotherapy. Three of these cases of SMZL continue to have normal hemoglobin and platelet count; however, leukocytosis and lymphocytosis persists.

## Diffuse Red Pulp B-cell Lymphoma

There were five cases (four males and one female); median age was 50 years (range, 40-58 years). Clinical details were available in two cases only. Hemoglobin and platelet counts were normal; however, both cases revealed lymphocytosis. One patient had abdominal lymphadenopathy. lactic dehydrogenase (LDH) was high in two and serum bilirubin was high in one case. Serum globulins were raised in one case. BM aspirate and biopsy revealed lymphocytosis in both cases (20 and 53%). Tumor cells were monomorphic, small to intermediate size, homogeneous nuclear chromatin with villous morphology. Tumor cells expressed CD19, CD20, CD22, and FMC7, while CD5, CD11c, CD25, and CD103 were negative in both the cases. Light chain restriction was seen in both cases. One case expressed CD5 and CD38, while another case expressed CD11c and was negative for FMC7. BM biopsy showed intrasinusoidal lymphoid aggregates in one and interstitial increase in lymphoid cells in another case. Splenic size was 12 cm and 18 cm. Grossly, the spleen was uniform, red, and congested in both cases. On microscopy, the capsule was thickened in one case. All five cases revealed a diffuse pattern of growth in the red pulp. Sheet of monomorphic cells filing the red pulp was a diagnostic feature. Tumor cells were round to oval with fine nuclear chromatin and moderate amount of cytoplasm [Figure 3]. Atretic white pulp follicle could be seen in three cases. Immunoblasts and plasma cells were occasional; one case had a prominence of hyalinized vascular channels. These five cases had been initially labeled as low-grade B-cell lymphoma of spleen (favoring a diagnosis of HCL in two cases and SMZL in another three cases). All expressed CD20 and BCL2. One case expressed CD5. Tumor cells were negative for CD23, cyclin D1, and CD10. Mib1 (Ki67) proliferation index ranged from 5 to 10%. Follow-up was available in two cases of DRP (3 and 5 years). One of them received six cycles of R-CHOP (Rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone) chemotherapy and is presently in remission.

## Follicular Lymphoma

There were three cases of splenic FL (two males, one female); median age was 51 years (range, 46-59 years). Two cases were grade 1, and one was grade 3 FL. Clinical details were available in

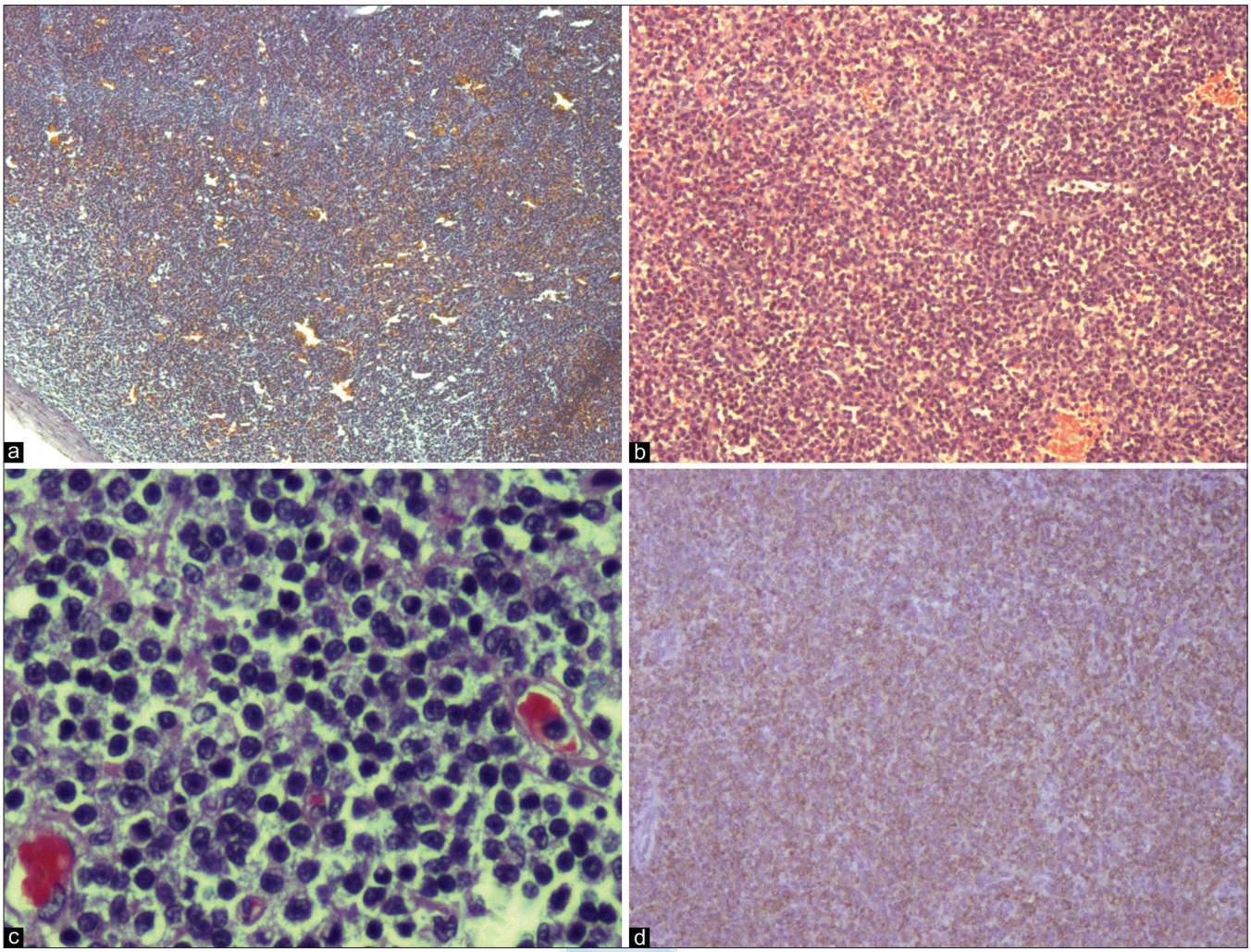


**Figure 3: (a-c) Splenic DRP lymphoma showing a diffuse pattern of monomorphic looking round tumor cells (H and E, ×20, ×100, ×400), (d) Splenic DRP lymphoma showing a diffuse pattern of monomorphic tumor cells expressing CD20, a pan B-cell marker (IHC stain - CD20, ×100)**

two cases only (FL Grade1, FL Grade 3). The patient with FL Grade 1 had lymphocytosis (47%), while FL Grade 3 had pancytopenia (with relative lymphocytosis) with hilar lymphadenopathy, high beta-2 microglobulin, and high LDH. Follow-up was available in both cases (4 months, 1 year). BM in both cases revealed lymphocytosis (18 and 53% lymphoid cells). BM biopsy revealed paratrabecular deposits of tumor cells. Tumor cells expressed CD19, CD20, CD79a, CD22, IgM, and light chain restriction. CD10 was positive in one case (FL, Grade 1), but was negative in FL Grade 3. IgD expression was not seen in any of the cases. There was one case where the splenectomy specimen was available (FL - Grade 1, size - 23 cm). Grossly, the spleen was uniform, red, and congested. Cut section revealed generalized tiny whitish nodules. On microscopy, all three cases revealed a nodular pattern in the white pulp [Figure 4]. Nodules revealed a mixture of small cleaved lymphoid cells along with centroblasts (varying proportion). Two of the cases were labeled as grade 1 and one case as grade 3, based on number of centroblasts. All cases expressed CD20 and BCL2. Tumor cells expressed CD10 in two of the three cases (FL, Grade 3 was negative). Tumor cells were negative for CD3, CD5, and CD23. Mib1 (Ki67) proliferation index ranged from 5 to 10% in both cases of FL, Grade 1. Patient with FL Grade 3 received only one cycle of chemotherapy (CHOP) and expired within a month. FL (grade 1) continues to have lymphocytosis.

### Hairy Cell Leukemia (Two cases)

Both were referral cases with a clinical impression of HCL. Both were males (age, 47 and 70 years). Splenectomy specimen was available in one case (splenic size was 23 cm). Grossly, the spleen was uniform, red, and congested. On microscopy, both cases revealed a diffuse growth pattern filling the red pulp. Sheets of monomorphic, round to oval cells with bean shaped nuclei, homogeneous nuclear chromatin were a predominant feature [Figure 5]. Tumor cells expressed CD20 and BCL2 and were negative for CD23 and CD10. Mib1 (Ki67) proliferation index ranged from 5 to 7%. Single case of HCL variant was a 40-year-old male presented as anemia, thrombocytopenia, and leukocytosis. Peripheral blood revealed 86% small to intermediate-sized lymphoid cells with clumped nuclear chromatin, with many tumor cells showing the presence

of nucleoli and short unevenly distributed cytoplasmic projections. Tumor cells were negative for CD11c and CD25, but expressed rest of the B-cell markers including FMC7 and CD103. BM biopsy revealed interstitial and intrasinusoidal deposits of tumor cells. Splenic size was 15 × 10 × 6 cm. Grossly, the spleen was uniform, red, and congested. On microscopy, it revealed a thick capsule. The pericapsular area revealed patent sinuses; however, the deeper areas revealed a diffuse red pulp involvement by the tumor cells. Tumor cells were round to oval, monomorphic, filling the red pulp with well-preserved white pulp. In addition, it showed sprinkling of eosinophils and an occasional megakaryocytes [Figure 6]. Tumor cells expressed CD20 and bcl2, but were negative for CD5, CD10, CD23, and cyclin D1. This patient received five cycles of 2-chlorodeoxyadenosine (cladarabine). The total leukocyte count became normal but the abnormal lymphoid cells persisted and patient developed anemia and thrombocytopenia. Splenectomy was performed but the tumor cells persisted in the peripheral blood, 6 months post-splenectomy.

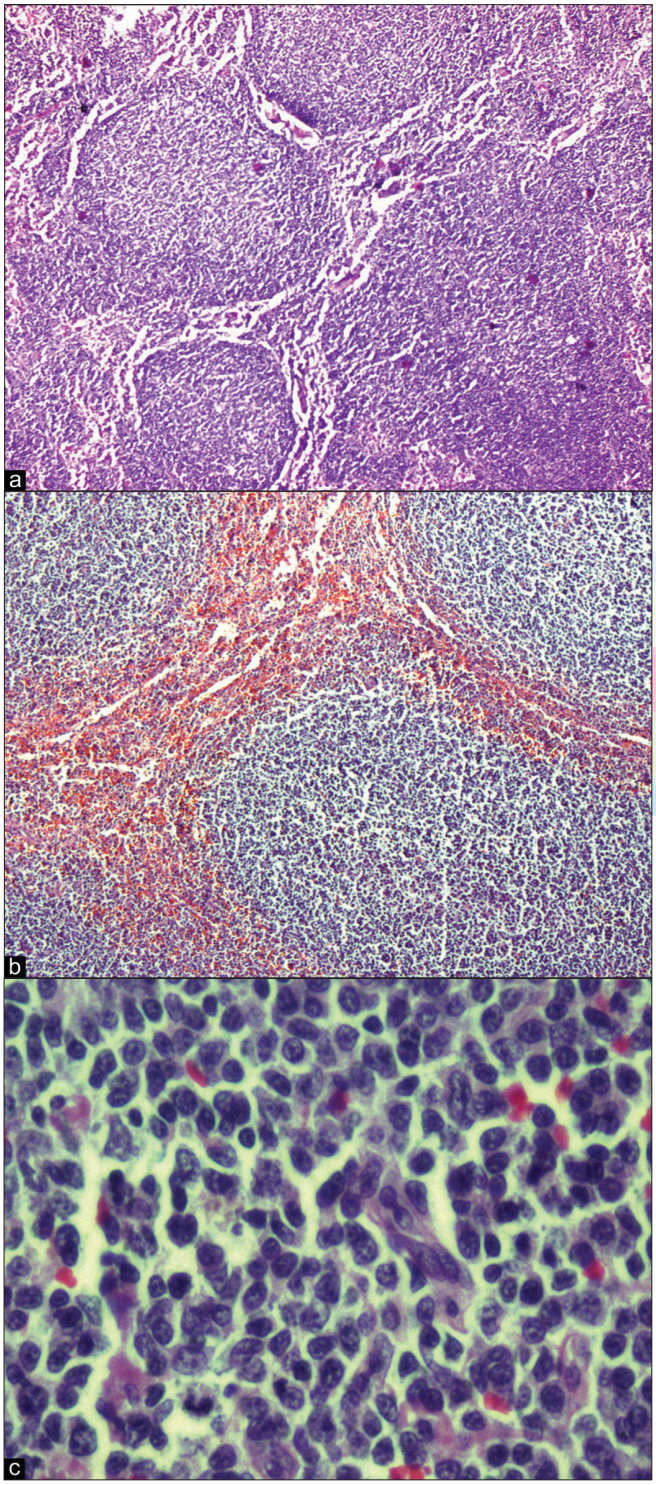


**Figure 4: (a,b) Follicular lymphoma, Grade 1 showing a nodular pattern of tumor cells (H and E, ×20, ×40) (c) Follicular lymphoma, Grade 1 showing a nodular pattern of tumor cells (H and E, ×400)**

### Diffuse Large B-cell Lymphoma

There were 14 cases of DLBCL (seven male, seven female patients); median age was 50 years (range, 40-70 years). Clinical details were available in five cases only. Left hypochondriac pain and abdominal discomfort or pain was seen in all cases. Two cases had diabetes mellitus. Serum alkaline phosphatase, beta-2 microglobulin, and LDH were raised in all five cases. Follow-up was available in these five cases (from 2 to 38 months). One case revealed pancytopenia. Fine needle aspirate cytology was available in two cases where a diagnosis of lymphoma was suspected. BM aspirates and biopsy was available in five cases. Aspirate revealed normocellular marrow in all five cases with lymphocyte count ranging from 7 to 22%. There was no evidence of involvement in the form of abnormal cells. BM biopsy also revealed a normocellular uninvolved BM in all five cases. FCM immunophenotyping available in one case was uninvolved. Splenectomy specimen was available in six cases only. Splenic size varied from 13 to 25 cm. Grossly, spleens were large with nodular protuberances in five cases. Cut section revealed generalized large size grayish white nodules. On microscopy, the capsule was thickened in two of the 14 cases. All cases revealed a predominant diffuse pattern with sheets of tumor cells involving the red pulp. Four cases focally revealed a nodular pattern comprising of mature-looking

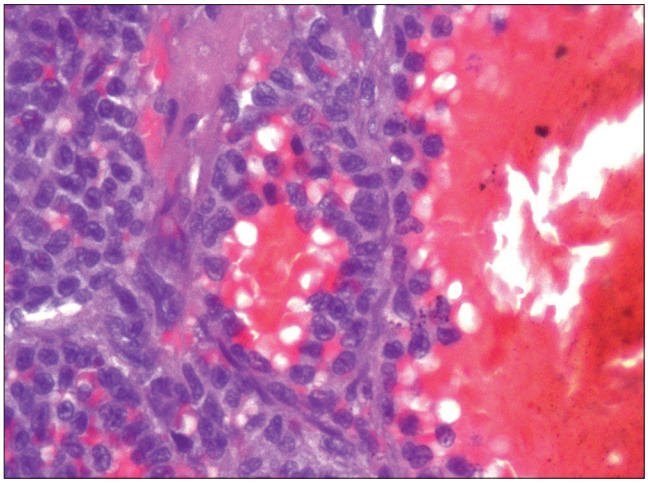

**Figure 5: Hairy cell leukemia showing red pulp infiltration by tumor cells with a prominence of blood lakes (H and E, ×400)**

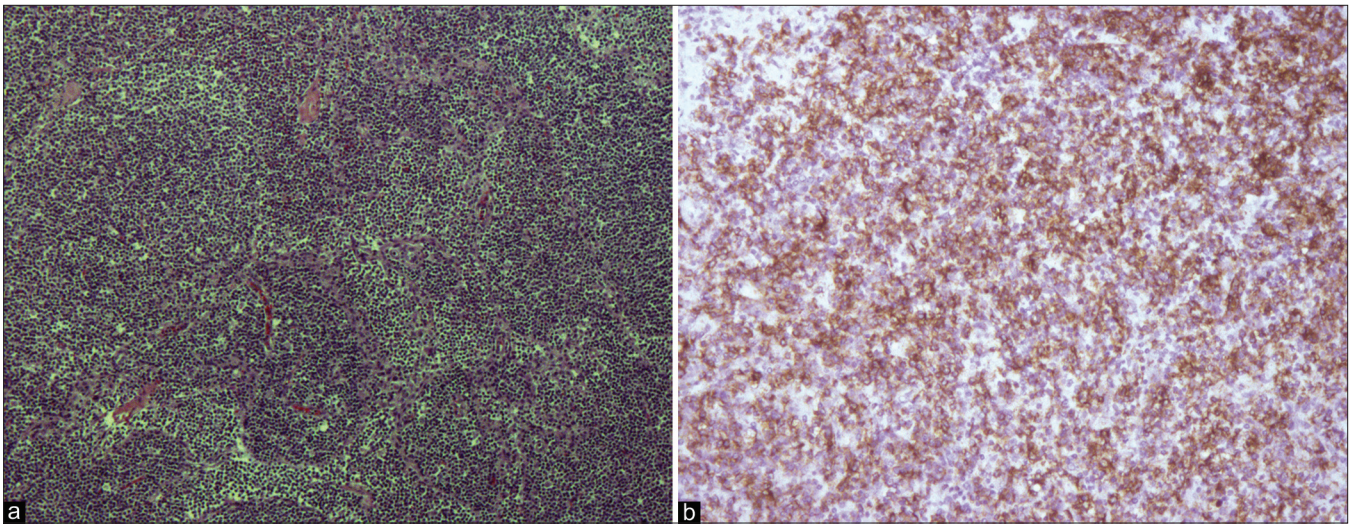


**Figure 6: (a) Hairy cell leukemia variant showing a diffuse pattern of involvement of red pulp by a monomorphic population of tumor cells (H and E, X 20), (b) Hairy cell leukemia variant showing CD20 expression in tumor cells (IHC stain, CD20 – ×100)**

lymphoid cells. There were two other cases which revealed focal areas resembling morphology of a low-grade lymphoma. Atretic white pulp could be demonstrated with CD20 stains. Tumor cells were large size, nucleolated, with moderate blue cytoplasm [Figure 7]. Multinucleated giant cells were seen in two cases. Two cases in addition revealed T-cell/histiocyte-rich nodules in the red pulp. There was a prominence of hyalinized vascular channels in one case. Tumor cells in all cases expressed CD20 and BCL2 and were negative for CD5, cyclin D1, and CD10. Mib 1 proliferation index available in three cases varied from 15 to 60%. No follicle dendritic cell meshwork infrastructure underlying the nodules could be demonstrated by staining for CD21, CD23, or CD35 antigens. Two patients had hilar splenic nodes, one involved by DLBCL, while the other one was a reactive lymphadenopathy. One had reactive supraclavicular lymph node, while another case had generalized abdominal lymphadenopathy (based on CT scan). Follow-up was available in four cases (2 months to 3 years). Two of these received six cycles of CHOP and are on follow-up. One patient took three cycles of CHOP and defaulted.

There were only two cases of splenic TCL. First one was a 25-year-old male, a case of HSGDTCL, in whom splenectomy was available (size, 23 cm). Microscopic examination revealed intrasinusoidal pattern of involvement. Tumor cells were intermediate to large size, fine nuclear chromatin, mitotically active. Tumor cells were highlighted with CD3 and CD43, but were negative for CD20, CD79a, Tdt, CD34, Mic2, CD30, and Alk1. BM biopsy revealed a hypercellular marrow with trilineage hematopoiesis, with scanty tumor cells within the sinusoids. Flow cytometry revealed expression of T-cell markers (CD3, CD7), CD56, and TCR gamma delta expression. Second case was of splenic TCL-NOS. It revealed intrasinusoidal pattern of involvement by small to intermediate lymphoid cells highlighted with CD3 and CD43, and intermixed with plasma cells and CD20 positive immunoblasts. This could not be further sub-typed as a complete immunophenotypic profile was not available in this cases including FCM immunophenotyping.

## DISCUSSION

Hematolymphoid neoplasms are systemic disease and it is difficult to label a splenic lymphoma as a PSL and most of these may be better defined as PdSL. PSL in the literature is often defined as generalized lymphoma with splenic involvement as the dominant feature and its diagnosis is based primarily on a splenectomy specimen. PSL as an entity is not recognized by the recent WHO classification of hematolymphoid neoplasm. The diagnosis is based on morphological and immunohistochemical analysis of a splenectomy specimen, and on a peripheral blood/BM immunophenotyping as a diagnosis of exclusion.[1-3] PSL characteristically involves the spleen, BM, and peripheral blood and most have simultaneous involvement of splenic hilar and/or abdominal nodes.[6] In one series, only 3% spleens removed because of splenomegaly were found to have lymphoma.[7] In another study, 3.4% of splenectomies with malignant lymphoma could be labeled as PSL.[8]

Of all 46 cases, one case of splenic DLBCL had abdominal lymphadenopathy and another case of HSGDTCL had hepatomegaly. Thus, these two cases though may be labeled as PdSL (total number - 46 cases), do not fulfill the criteria of coming under umbrella of PSL (total number - 44 cases). Ours being a tertiary care cancer center, PSL constitute approximately 0.6% of all new cases of lymphomas, an incidence lesser than 1% as reported elsewhere.[1,2] We did not include those splenic lymphomas which presented as a leukemia, where splenectomy was not performed. Common subtypes of PSL in our series were SMZL (41%) followed by DLBCL (30.4%), DRP (10.8%), FL (6.5%), HCL (4.3%), HCL-v (2.1%), and TCL (4.3%). We recently had a case each of mantle cell lymphoma and Hodgkin's disease (not included in this study), as also reported by others.[1-3,5,9]

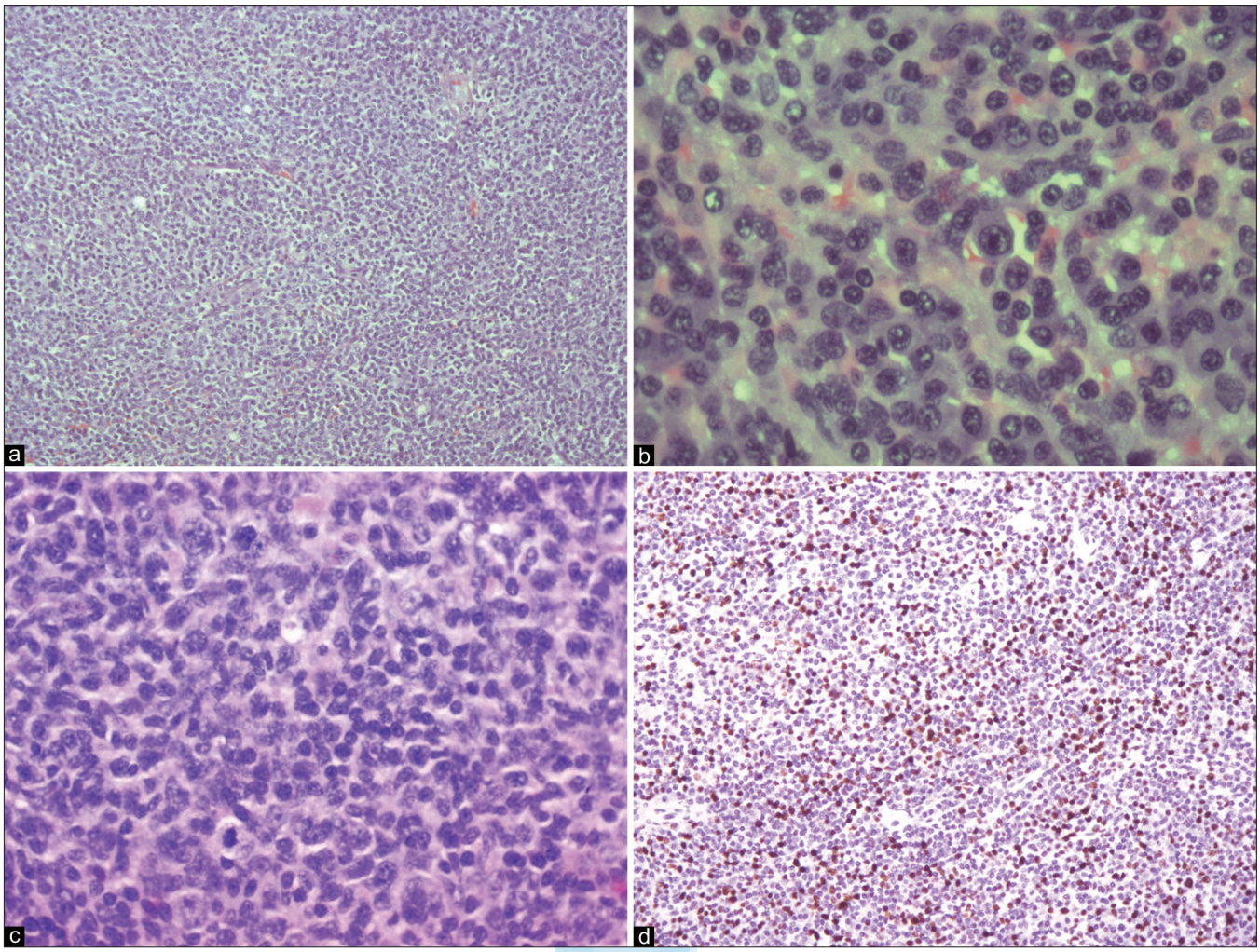


**Figure 7: (a-c) Splenic diffuse large B cell lymphoma showing sheets of large sized tumor cells (H and E, ×40, ×400, ×400), (d) Splenic diffuse large B cell lymphoma showing tumor cells expressing a high Mib 1 proliferation index of 50% (IHC, Mib1 proliferation marker, nuclear stain, ×100)**

SMZL was the commonest subtype, as reported by others.[1,2] It is a disease of the elderly.[1,2] Median age in our series was 53 years (range, 41-83 years). Many patients with SMZL are asymptomatic at diagnosis. All our cases presented with gradually progressing abdominal swelling and revealed lymphocytosis on smear examination, confirmed as SMZL on morphology and immunophenotyping. Splenectomy was performed for therapeutic purposes, mainly because of the discomfort. Most cases of SMZL may be diagnosed without recourse to splenectomy, as observed in present study.[10] There is a considerable histologic, immunohistochemical, and molecular heterogeneity of SMZL, indicating an origin from the diverse resident B-cell populations of the normal splenic marginal zone. All our cases had involvement of peripheral blood and/or BM, and FCM immunophenotyping was diagnostic. Palpable lymphadenopathy is rare but splenic hilar lymph nodes are commonly enlarged. Six of the seven cases had lymphadenopathy, involving splenic hilar nodess (4), and supraclavicular nodes (1) and generalized abdominal lymphadenopathy (1). Anemia and thrombocytopenia are common at presentation. Serum monoclonal M band may be seen in one-third of the patients. Though serum globulins were raised in six of these seven cases, serum electrophoresis was not available. Autoimmune cytopenia (anemia, thrombocytopenia) are common in SMZL.[11] Anemia and thrombocytopenia was seen in six and two of the total seven cases, respectively. The prevalence of HCV infection is reported to be as high as was 68% among PSL.[12] However, none of our cases had HCV infection. Common BM patterns seen in our series were nodular and interstitial. Intrasinusoidal pattern was seen in one case only (highlighted by CD20 stain), unlike as described in the literature where the intrasinusoidal is the commonest and the classical pattern of involvement.[13] The immunophenotype of SMZL has been evaluated by both flow cytometry[1,14,15] and IHC.[16,17] FCM analysis demonstrated expression of the pan B-cell antigens CD19, CD20, CD22, and CD79b, with light chain restriction (moderate to strong intensity). Most cases express both IgM and IgD, while a minority of cases appears to be IgM+IgD- or IgG+.[14,15] IgM was expressed in all our cases and IgD was expressed weakly in two cases only. CD5 expression is documented in up to 20% of SMZL.[10] CD5, CD23, and cyclin D1 immunostaining were negative in all our cases of SMZL. Cytogenetic and/or molecular genetic abnormalities

can be detected in approximately 80% of patients, although there is no single abnormality that is present in all cases. Recurring clonal abnormalities include Trisomy 3, deletions of 7q and 17p, and translocations involving the immunoglobulin gene loci.[1,18,19] Conventional cytogenetics was performed in three cases (normal studies in two and Trisomy 12 in one case). Prognostic factors are poorly defined and only loss or mutation of the p53 gene is consistently associated with a poor outcome.[1,18,19] None of our patients received any chemotherapy and all are on regular follow-up (maximum up to 4 years). The reported median survival times range from eight to 13 years, depending on whether the studies only include patients who have undergone splenectomy,[20] or who have splenomegaly[21] or have greater than 10% circulating villous lymphocytes.[22] In another study published from our laboratory, SMZL constituted approximately 18 cases (5%) of a total of 356 cases of mature B-cell NHL cases which presented as leukemia. These cases were primarily diagnosed on peripheral blood/BM aspirate examination and splenectomy was performed only in nine of these cases.[23] It has been proposed that SMZL, nodal MZL, and even Waldensrom macroglobulinemia (WM), all with a classical CD19+CD20+CD5-CD10-CD23- immunophenotype, may form a continuous spectrum of disease characterized by similar morphological and phenotypic characteristics which are only differentiated on the basis of clinical features such as pattern of disease distribution.[24,25]

DLBCL may begin initially at extranodal sites in 40% cases, commonly in gastrointestinal tract, bones, testis, spleen, Waldeyer's ring, etc.[4] However, primary DLBCL of spleen are rare. When disease transformation occurs in SMZL, the histological appearances are those of diffuse large B cell lymphoma (DLBCL).[26-28] DLBCL has been reported in 22 to 33% of PSL and have a poor outcome.[8] It was second common subtype (30%) of PdSL in our series. Two cases in addition revealed T-cell rich/histiocyte-rich nodules in the red pulp, as reported by others.[29] Transformation of a low-grade B-cell lymphoma to a DLBCL is well known.[27] Of the total 14 cases of DLBCL, six had a focus of low-grade lymphoma, thereby suggesting a transformation of a low-grade lymphoma to a large B-cell lymphoma. None of our DLBCL had BM involvement, in contrast to other studies, where BM biopsies have revealed intravascular/intrasinusoidal lymphomatous marrow infiltration.[30] Splenectomy followed by combination chemotherapy results in excellent long-term survival in PSL.[31] Two of the four cases have responded well to six cycles of CHOP chemotherapy.

Splenic B-cell lymphoma/leukemia unclassifiable as per recent WHO classification includes DRP and HCL-v. Strict diagnostic criteria are not well established. Entities like HCL and HCL-v are primarily diagnosed based on peripheral blood/BM examination and splenectomy is not required for its diagnosis, while newer entities like DRP require splenectomy specimen for their diagnosis. DRP shows a diffuse pattern of involvement of the red pulp by monomorphous B-cells. Peripheral blood may show villous lymphocytes and BM may show intrasinusoidal pattern of infiltration. It constituted 12% of all PdSL in our series. There are only occasional reports of DRP in the literature.s[4] It is associated with thrombocytopenia, leucopenia, and a massive splenomegaly.[4] One case of DRP expressed CD5. Close morphological differential is HCL as seen in our series.[4] The clinical picture, morphology with FCM immunophenotyping was essential in the diagnosis. Splenic TCLs are extremely rare and common subtypes include HSGDTCL.[32] We had only two cases of PdSL of T-cell phenotype, one case of HSGDTCL with BM involvement (intrasinusoidal pattern) and the other case of TCL-NOS which could not be further characterized due to lack of material and the clinical information.

There were a few lacunae in this study. Adequate workup was available in 19 cases only; referral cases had paraffin blocks with sketchy clinical details only. Stains for IgM and IgD were available in few cases only. Annexin A1 was not available for immunophenotyping. Molecular genetics was done in a few cases only. Survival curves could not be established due to small number of cases and incomplete workup available. We did not include those cases of SMZL where diagnosis was established on BM or peripheral blood immunophenotyping and splenectomy was not available. As seen in most medical centers, hematopathology laboratory with its two components of solid and liquid hematopathology is distributed in different departments of pathology and hematology, respectively. Present study shows data from the department of pathology where only solid tissues (other than BM, peripheral blood, and other fluids) are studied. SMZL and HCL constitute another 5% each of all the chronic lymphoproliferative disorders presenting as leukemia in our center (where splenectomy is not performed).[23] Combine data from both the laboratories reveal that splenic lymphomas constitute approximately 0.96% of all the hematolymphoid neoplasm presenting in our cancer center, a figure same as seen in the western literature.[1,2] Apart from common types of splenic lymphomas, we had splenectomy done for cases of Waldenstrom's macroglobulinemia, Hodgkin disease, and mantle cell lymphoma in the years 2009 to 2010 (one case each, not included in this study).

Though hematolymphoid neoplasms are systemic diseases, PSL described as generalized lymphoma with splenic involvement as the dominant feature may be better classified as PdSL, entities not recognized by the 2008 WHO classification of hematolymphoid neoplasm. All our cases of Splenic lymphoma (except DLBCL subtype) had BM involvement at presentation (stage IV disease). Thus, PdSL may be a more apt term for such lymphomas instead of PSL. PSL (and PdSL) constitutes approximately 0.6% of all new cases of lymphomas seen in our histopathology laboratory (0.96% if include cases of splenic lymphomas presenting as leukemia). Approximately 96% of PdSL are of B-cell phenotype. Common subtypes were SMZL (41%), DLBCL (30.4%), and DRP (10.8%). BM involvement was commonly seen in SMZL (nodular pattern) and DRP (intrasinusoidal and interstitial). None of DLBCL involved the marrow. Higher percentage of DLBCL in our series could be due to late presentation of our cases. Newly

defined entities like DRP and HCL had a similar diffuse red pulp pattern of splenic involvement. Clinical profile, CBC assisted with morphology and a battery of immunomarkers on splenectomy, as well as BM aspirate specimens help in a definitive diagnosis. Common immunophenotypic markers (LCA, CD20, CD3, CD5, CD10, CD23, FMC7, CD43, IgM, IgD, CD22, kappa and lambda light chains, CD79a, Bcl-2, and cyclin D1) help in narrowing down the diagnosis of these morphologically similar looking neoplasms. Most of the splenic lymphomas are low-grade type, except DLBCL and the TCLs. Splenectomy was required for diagnostic purposes mainly in DLBCL, while rest of the cases could be diagnosed based on peripheral blood/BM involvement (with FCM immunophenotyping). It is crucial to diagnose HCL as it has a definitive treatment protocol. Others like SMZL, DRP, and HCLv are managed with observation in asymptomatic cases, and splenectomy is considered as a palliative therapy for patients not responsive to immunotherapy (rituximab) with or without chemotherapy.[33]

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