

IGH/BCL2 Status Better Predicts Clinico-Pathological Behavior in Primary Splenic Follicular Lymphoma than Histological Grade and Other Molecular Markers

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ABSTRACT: Splenic lymphoma may be primary or secondary. Primary splenic lymphoma's are rare and usually of follicular cell origin representing <1% of Non-Hodgkin's Lymphoma's. Most are secondary with 35% representing Marginal Cell sub-type with the rest being Diffuse Large B-Cell Lymphoma's. Unlike the uniformly aggressive clinical course of Diffuse Large B-Cell Lymphoma's, biological behavior of Primary Splenic CD10-Positive Small B-Cell Lymphoma/Follicular Lymphoma remains less well defined. We present here a solitary splenic mass confirmed as Primary Splenic CD10-Positive Small B-Cell Lymphoma/Follicular Lymphoma after a diagnostic splenectomy. Biopsy revealed monomorphic small lymphoid cells with low grade mitotic activity. Flow cytometry showed a lambda restricted population of B-Cells displaying dim CD19 and CD10. The cells were negative for CD5, CD11c, and CD103. FISH was negative for IGH/BCL2 fusion unlike nodal Follicular Lymphoma's which are usually positive for this translocation. Evidence from this case and a review of literature support the finding that Primary Splenic CD10-Positive Small B-Cell Lymphoma/Follicular Lymphoma is less likely to have the classic IGH-BCL2 fusion and the associated chromosomal 14;18 translocation. This profile is associated with less aggressive clinical behavior even when histopathology represents a high-grade pattern. In such cases splenectomy alone is adequate for localized disease when negative for IGH/BCL2 fusion regardless of histological grade.

KEYWORDS: Lymphoma, florescence in situ hybridization, immunohistochemistry, molecular pathology

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Introduction

Follicular Lymphoma (FL) is a sub-type of indolent B-cell lympho-proliferative disorders arising from transformed follicular center B-cells. It is the second most common of the Non-Hodgkin's Lymphoma's (NHL) after Diffuse Large B-Cell Lymphoma's (DLBCL), accounting for 35% of NHL's. However, it represents 70% of all indolent Non-Hodgkin's Lymphoma's. Most cases present as generalized lymphadenopathy with extra-nodal presentations representing <10% of all cases. Primary Splenic Follicular Cell Lymphoma (PSFCL) is even rarer and represents <1% of all Non-Hodgkin's lymphoma's.¹ Secondary involvement of the spleen is more common, and this is usually from FL (20%)² or Mantle Cell Lymphoma (35.4%).³ Other low-grade lymphoma's that may infiltrate the spleen include Splenic Marginal Zone Lymphoma (SMZL), Lymphoplasmacytic Lymphoma and Splenic Red Pulp Lymphoma. With the more common nodal FL, immunohistochemistry in almost all cases is positive for cell surface CD10, 19, and 20 markers. It is also characterized by the t(14;18) (q32;

q21) translocation between the IGH gene at 14q32 and the BCL2 gene at 18q2113. This causes juxtaposition of the BCL-2 gene with the IGH promoter causing dysregulated expression of BCL-2 which is most likely the initial oncogenic process in FL. Nevertheless, it is likely that multiple genetic events are required for the development of FL since over-expression of BCL-2 alone can be seen in normal individuals as well.⁴ These may include gain of function mutations in the H3K27 methyltransferase EZH2 which has been described in approximately 27% of cases⁵ as well as upregulation of genes including ETV1 along with altered T- Cell function in the tumor milieu.⁶

Also, approximately 6% of FL's could be positive for BCL-6 mutation which may represent another oncogenic mutation.⁷ NHL's presenting as isolated splenomegaly is uncommon. The most common NHL to present like this is the DLBCL subtype. Indolent lymphoma's including the MZL subtype as well as FL's tend to present with gradually progressive lymphadenopathy, and it is only in the advanced stages that they may be associated with splenomegaly. And even when there is splenic involvement, it usually takes the form of a diffuse splenic infiltrative process. Isolated presentation therefore, of FL as a solitary mass lesion is unusual.⁸ In a review of 17 cases of solitary

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splenic lymphoma's, 9 were DLBCL's followed by 4 SMZL's. Only 2 were considered as probably FL's and 1 being Splenic Red Pulp Small B-Cell Lymphoma.

Hodgkin's Lymphoma was also uncommon with only 1 of 17 in this case series.⁹ Considering this rarity, histologic, immuno-histochemical criteria as well as clinical behavior of most splenic lymphoma's excepting for DLBCL and SMZL subtypes remain unclear.¹⁰ We present here a case of a solitary splenic mass detected on imaging studies and confirmed as Primary Splenic **CD10-Positive Small B-Cell Lymphoma**/Follicular Lymphoma after a diagnostic splenectomy. Evidence from this case and a review of literature support the finding that extra-nodal FL's including PSFCL's are less likely to have the classic IGH-BCL2 fusion and the associated chromosomal t(14;18) translocation. And this molecular and cytogenetic profile is associated with a less aggressive clinical behavior even when the pathology is high-grade.

Case Presentation/Literature Review

A 58-year-old female presented with a history of left upper quadrant abdominal pain for more than 2 months. This was accompanied by un-intentional weight loss documented as 10lbs. and night sweats.

Medical history was significant only for Hypertension, Atrial Fibrillation and Anxiety disorder.

Physical examination showed palpable splenomegaly extending 3 cm below the costal margin. The patient was initially evaluated at an outlying facility with a CT of the abdomen showing mass lesions in the spleen as well as one near the lesser curvature of the stomach. Upper GI Endoscopy was done with endoscopic ultrasounds. There was a well-defined splenic mass measuring approximately 54 mm × 43 mm along with what appeared to be a splenic hilar node measuring 8.1 mm × 6.7 mm. There was also a sub-carinal node measuring 17.8 mm × 10.6 mm. FNA of all 3 masses were completed. The splenic hilar mass FNA however did not reveal any lymphoid tissue while the sub-carinal FNA showed non-caseating granulomatous tissue only. Comparison of the initial CT image with subsequent staging PET CT indicated a possible artefact in the area of the left lobe of the liver adjacent to the lesser curvature of the stomach which had initially been read as a mass lesion involving the gastric antrum. There was no concerning PET avid area to indicate disease elsewhere. Da Vinci robotic splenectomy was subsequently performed. Exploration of the abdominal cavity did not reveal any enlarged lymph nodes and the spleen was removed without fragmentation. It measured 17 cm × 11 cm × 6 cm and weighed 573 g. The outer surface showed an area of white discoloration measuring 6.5 cm × 0.5 cm. The remainder of the outer surface was covered with a normal appearing glistening gray capsule. The cut surface showed a white, firm well circumscribed nodule at the periphery of the spleen measuring 1.5 cm × 1.3 cm × 1.3 cm along with another centrally located similar lesion measuring



Figure 1. Gross surgical splenectomy specimen showing an area of whitish discoloration measuring 6.5 cm with the rest of the outer surface being covered with a normal appearing glistening gray capsule.

Table 1. Hematological and metabolic profile.

PARAMETER	VALUE
WBC	7.2 × 1000/mm ³
RBC	4.35 mil/mm ³
Hgb	13.2 g/dL
MCV	88.0 fl
MCH	30.4 pg
MCHC	34.6 g/dL
RDW	14.0%
Sodium	138 meq/L
Potassium	3.6 meq/L
Chloride	102 meq/L
Carbon Dioxide	28 meq/L
Calcium	8.8 mg/dl
Glucose	163 mg/dL
Urea Nitrogen	9 mg/dL
Creatinine	0.66 mg/dL
LDH	173 IU/L

5 cm × 4 cm × 5 cm. The remainder of the cut surface was beefy tan, solid, and mostly homogenous in appearance (Figure 1). Complete blood count and basic metabolic profile showed the following (Table 1).

Results of cytology and microscopic examination showed a monomorphic population of lymphoid cells of small size, admixed with a minor population of medium sized lymphocytes (Figure 2). Only few of these lymphoid cells appeared cleaved and the nucleoli were either inconspicuous or small.

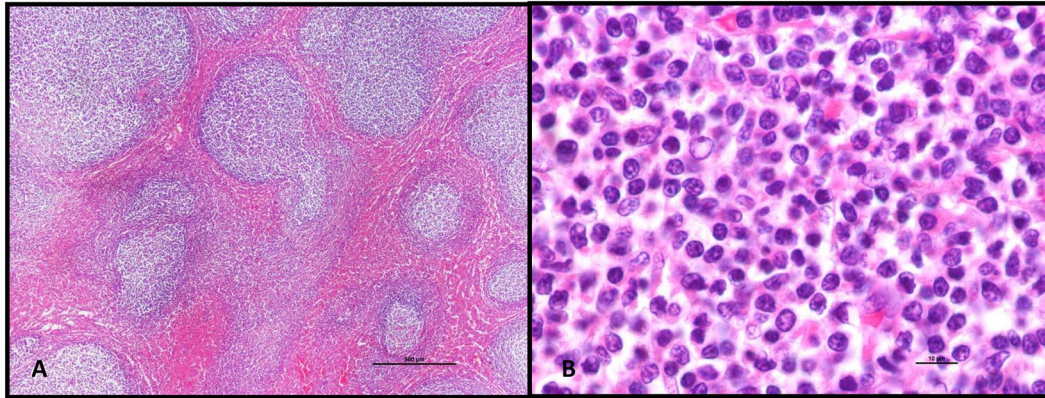


Figure 2. (A) Low power magnification of splenic mass section reveals non-uniform nodular involvement of the white pulp (Hematoxylin-eosin stain, original magnification $\times 40$). (B) High power magnification of a nodule reveals the lymphocytic tumor infiltrate with a homogeneous uniform population of small cells with irregular nuclear outlines (Hematoxylin-eosin stain, original magnification $\times 1000$).

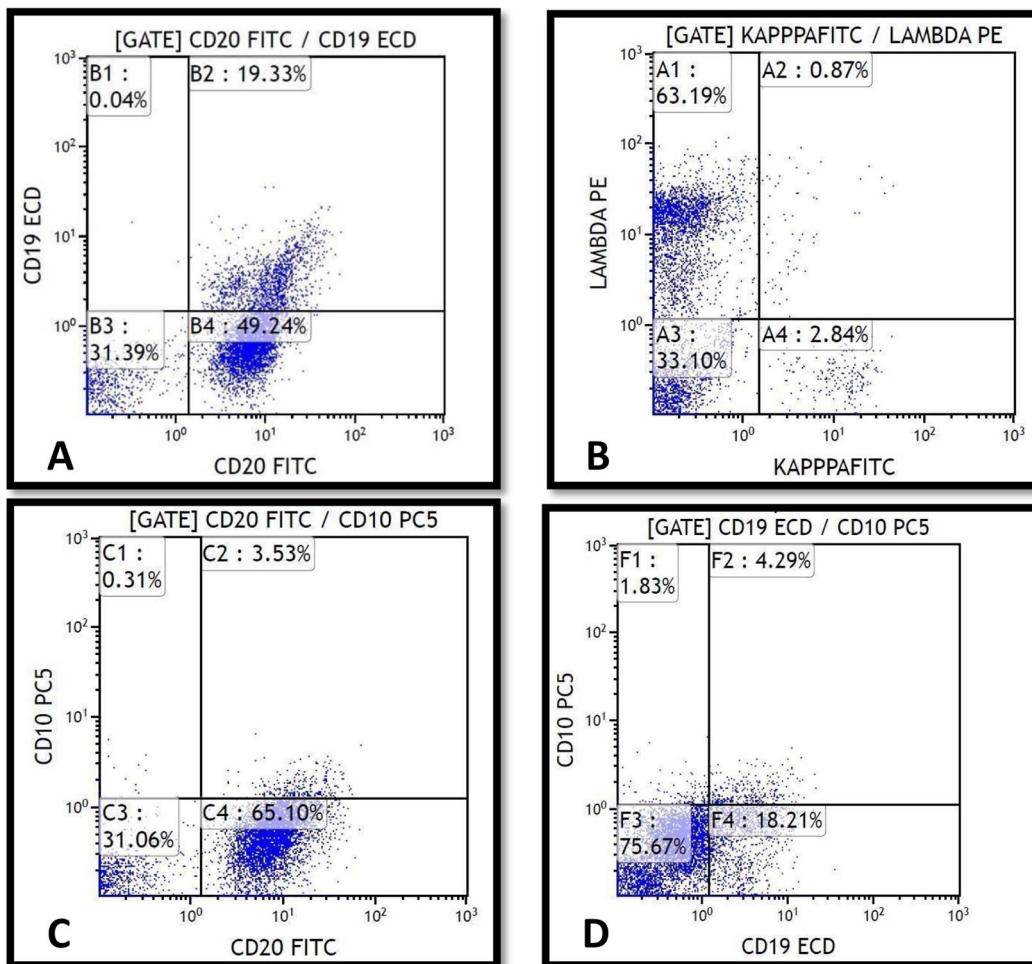


Figure 3. Flow-cytometric characterization of clonal B-cell population in PSFCL: (A) CD20 bright with moderate intensity and CD19 positive (dim). (B) Lambda light chain bright. (C and D) CD10 positive(dim).

Histological grade was low. There was no brisk mitotic activity, necrosis, or sheets of large lymphoid cells. Flow cytometry results revealed a lambda restricted monotypic population of B-Cells displaying dim CD19 and CD10 (Figure 3). The neoplastic lymphoid cells were negative for CD5, CD11c, and CD103.

Cytogenetic evaluation by the G-banding technique and Fluorescence In Situ Hybridization (FISH) studies were done using the LSI IGH dual color break-apart probe and the LSI IGH/BCL2 dual color fusion probe.

Cytogenetics showed one normal female diploid cell line. FISH studies were negative for IGH/BCL2 fusion but did

indicate the presence of an extra green IGH signal consistent with disruption of the IGH locus most likely involving a translocation gene other than BCL2. FISH was however positive for chromosome 13q deletion at D13S319 locus (29% of cells) and deletion of 6q telomere region (50% of cells). These findings are consistent with a Stage 1 low grade Primary Splenic CD10-Positive Small B-Cell Lymphoma/Follicular Lymphoma with a FLIPI score of 0 (Age < 60, Hgb > 12, LDH normal, Stage not III/IV and lymph node sites not > 4). Repeat PET CT done 4 years later was negative for any evidence of disease recurrence.

Discussion

Follicular lymphoma is a low-grade lymphoproliferative disorder which usually presents with generalized lymphadenopathy. However, as the disease advances, extra-nodal involvement can occur and this often involves the bone marrow, skin, and duodenum. Splenic involvement occurs only much later and in advanced stages.

Primary Extra-Nodal FL however can originate in any organ, but appears to commonly arise in the Spleen, Salivary glands, Skin, Duodenum, Testes, and the Ovary. Studies have shown that approximately 13% of female genital tract, 17% of Testicular and 38% of Duodenal FL's are Primary FL's in these organs.¹¹⁻¹⁴ Ozsan et al looked at 16 cases of Primary Ovarian FL's and found that those that were BCL-2 negative and did not have the conventional IGH-BCL2 translocation tended to have higher grade and lower stage while those that were BCL-2 protein expressing and positive for IGH-BCL2 translocation were of lower grade and higher stage. In this cohort all cases that were BCL2+ were of Grade 1 only. Three of the seven BCL-2 protein negative cases were Grade 2 and the rest all Grade 3A. Interestingly higher-grade cases (4 of 7 in this group) tended to remain localized as Stage IE as against 6 of 9 that were Stage III in the BCL2 protein positive low-grade group.¹⁵ The paper by Goodlad et al looked at 15 cases of non-cutaneous extra-nodal FL and showed that only 2 of the 14 cases of extra-nodal FL had the t(14;18) translocation compared to 9 of 16 nodal FL's which were positive for t(14;18) ($P < .01$). This paper also compared outcomes with 87 cases of early-stage nodal FL's. 13/15 cases were disease free at the end of follow up compared to only 49/87 in the Stage I nodal FL group ($P < .02$). This paper thus points out the propensity of a subtype of FL that is negative for t(14;18), has a favorable prognosis and is more likely to arise in extra-nodal sites.¹⁶ In another study by Mollejo et al, 20 of the 32 cases of splenic follicular lymphoma's had absent or only dim expression of BCL2 while the rest had strong homogeneous BCL2 staining. Incidence of t(14;18), CD10+ and low histological grade were significantly more common in the BCL2 positive cases (50% vs 7.1%). BCL2 protein negative cases tended to have higher grade (65% vs 8.3%, $P .002$). CD10+ was nearly twice as likely in the BCL2 + cases (81.8% vs 45%). Higher Ki67 staining was strongly associated with the cells being BCL2

protein negative. Bone marrow infiltration was detected in only 9 of 18 cases (50%) of BCL2 protein negative cases while it was positive in 80% of BCL2 protein positive cases. Clinical follow up though did not show any significant differences in OS at 5 years.

It is unclear if this resulted from the cases being more heterogeneous or the disproportionate use of systemic chemotherapy. 77.8% of BCL2 + cases did receive systemic chemotherapy as against only 27.8% of BCL2 protein negative patients even if bone marrow involvement was positive in 50% versus 80% (BCL2+) cases. In other words, less of the BCL2 protein negative patients were considered for chemotherapy.¹⁷ In the Weinberg study too, fewer t(14;18) negative cases showed positivity for BCL2. Clinical outcomes are probably difficult to compare in the Weinberg et al group because of the inclusion of Primary Cutaneous Follicular Lymphoma's (PCFL) which are known to have unusually favorable outcomes. These outcomes still appear to favor BCL2 positive cases when analyzed without PCFL's, even if this analysis was not stage defined.¹⁸ Also, unlike the paper by Goodlad et al, the Weinberg data doesn't limit the series to Stage I FL's. Regardless the discordant association between BCL-2 and Stage was supported by another Weinberg et al paper which found that presence of IGH/BCL2 translocation was associated with higher disease stages.¹⁹ Surface markers appear to be distinct as well. In the paper by Weinberg et al, Extra-Nodal FL was mostly CD10 negative.¹⁸ This study looked at 71 cases presenting in extra-nodal non-cutaneous organs with no lymph node involvement (IE) or only minimal lymph node involvement (IIE). As to whether these differences translate to distinct morphological subtypes remain unclear. The Weinberg paper indicated that BCL2 negative extra-nodal FL tended to have a diffuse growth pattern. In a series of 32 patients¹⁷ all cases showed a micronodular pattern centered on the white pulp. Histological grade was low (grade 1 or 2) in 18 of the 32 cases and high grade in the rest. And consistent with prior findings, BCL-2 negative cases tended to have a significantly greater proportion of higher grades (65% vs 8.5%). CD10 was positive in most cases that were BCL-2 positive (81.8%). Marrow involvement too was consistent with prior findings, being more common with BCL2 protein expressing cases. Only 9/18 (50%) of the BCL-2 negative cases had marrow involvement as against 8 of 10 (80%) of the BCL-2 positive cases. In this series, 9 cases had isolated splenic involvement only and they were all BCL-2 negative, without t(14;18). This study appears to indicate that isolated Splenic FL's are more likely to be BCL-2 negative with a higher histological grade. Interestingly this pattern is common in other extranodal FL as well including those of the testis²⁰ and skin.²¹

This pattern of PSFCL being more likely to be negative for BCL2 and t(14;18) translocation seems to conflict with the findings from a series of 16 cases studied by Howard et al. In this paper, all 16 cases of Primary Splenic Lymphoma's studied were positive for CD10 and BCL-2 protein expression.²² However,

within this group splenectomy was done for additional staging in 1 case and as part of work up for cytopenia's in 6 cases, the latter group indicating the presence of a systemic involvement. This leaves 5 cases where splenectomy was done after an enlarged spleen was detected on routine imaging, 3 cases where there was spontaneous splenic rupture and 1 case where the finding was incidental on a distal pancreatectomy. All of this makes it difficult to confirm the cases which represent PSFCL's as against secondary involvement. Regardless when clinically followed up for a median of 20 months, OS was favorable with Primary Splenic Lymphoma with only 1 patient having died of the disease. Interestingly the benign nature of isolated splenic lymphoma's was suggested much earlier by Brox and Shustik in as early as 1993.²³

The case presented here is characterized by dim CD10 expression and disruption of the IGH locus. The latter is evidenced by the presence of an extra green IGH signal consistent with the presence of an alternate IGH fusion gene likely involving a translocation gene other than BCL2. Minor breakpoints involving the BCL2 gene have been documented and so far, include- 1cr, 3'BCL2 and 5' mer (Weinberg, AJCP, 2009). In this case the molecular and cytogenetic pattern is consistent with the low-grade histological presentation.

Conclusions

Primary Splenic CD10-Positive Small B-Cell Lymphoma/Follicular Lymphoma has distinct immunohistochemistry, molecular and cytogenetic features that favorably influence its clinical behavior. Clinically non-aggressive pathophysiology is maintained even despite having high grade histology. And higher grades are often associated with lack of CD 10, less BCL2 protein expression as well as absence of the conventional IGH-BCL2 fusion gene. Tumors which are CD10 positive or dim and have either the classic IGH-BCL2 fusion gene or its variant tend to be lower grade. It therefore appears that in both these pathological subtypes of PSFCL's, there are mitigating factors that make even the higher histological grade clinically indolent. Therefore, Primary Splenic CD10-Positive Small B-Cell Lymphoma/Follicular Lymphoma is likely a distinct clinicopathological entity that can be managed less aggressively with splenectomy alone when it presents as a Stage IE disease.

Author Contributions

CV, WL, HS: Drafting of the manuscript. CV, WL: Acquisition and interpretation of data. CV, NG, NK, EA: Critical revision of the manuscript. HS, RB: Technical and material support.

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