Case Report

In situ follicular neoplasia/lymphoma: Three illustrative cases exemplifying unique disease presentations

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ABSTRACT

We report three elderly patients with follicular lymphoma *in situ* (FLIS) each highlighting a unique pattern of disease presentation and progression. The first patient had incidentally detected FLIS with peripheral blood spill and yet had an 11-year uneventful follow up. The second patient with an overt follicular lymphoma (FL) developed high-grade transformation in jejunum with FLIS extensively involving the Payers patches. The third patient had a FLIS but that qualified as higher grade and was treated in spite of lack of overt FL mainly because of higher grade and patient subsequently did develop overt FL. The first case of typical FLIS confirms that peripheral blood spill does not connote poor prognosis in FLIS, the second case illustrates that FLIS may colonize mucosa-associated lymphoid tissue as part of homing in process of a disseminated FL and the third case validates the aggressive nature of high-grade FLIS.

KEY WORDS: Follicular lymphoma, follicular lymphoma *in situ*, non-Hodgkin's lymphoma

Access this article online
Website: www.ijpmonline.org
DOI: 10.4103/0377-4929.97878
Quick Response Code:

INTRODUCTION

The concept of "*in situ*" lymphoma is similar to "*in situ*" carcinoma and the lesions consist of neoplastic lymphoid cells that proliferate in the place that is occupied by their normal counterpart without invasion of surrounding lymphoid structures.[1] Follicular lymphoma *in situ* (FLIS) is one of the best described examples of *in situ* lymphomas. The concept of FLIS was born in 2002 when Cong *et al* documented few patients with reactive nodes showing germinal centers that carried the IGH/Bcl2 translocation like follicular lymphoma (FL); however, many patients were asymptomatic and they put forward this as an early event in lymphomagenesis.[2]

The concept has since evolved from its recognition to its inclusion in the WHO 2008 classification[3] and the European Association of hematopathologist (EAHP) meeting held in Uppsala, Sweden in 2010 focused specifically on such events involved in early lymphomagenesis. Though originally some authors used the terms partial involvement of a node by FL,[4] and lesions of FLIS purely confined to the germinal centers interchangeably, FLIS is now restricted to the latter cases only.[5] Also the present definition of FLIS includes patients with centrocyte-like cells only carrying the bcl2 translocation but restricted to the follicles and a low MIB1 labeling. In the EAHP meeting in Sweden in 2010, however, higher grade FLIS were discussed and some were reported to have uneventful follow up. Though guidelines for such higher grade FLIS are not defined, the trend is to treat them as overt or manifest FL. The present report seeks to highlight three unique situations of occurrence of FLIS keeping the above evolution in mind.

CASE REPORTS

Case 1

A 55-year-old male patient presented in the year 1997 with lymphocytosis in peripheral blood detected incidentally during an episode of fever. The patient was referred to our institute for further management. A bone marrow examination revealed scanty clusters of atypical small lymphoid cells that marked with CD10 and CD20 and a diagnosis of Stage IV FL was made. A routine CT (computerized tomography) of thorax abdomen revealed that patient had no significant lymphadenopathy except for three to four tiny nodes less than 1 cm in both axilla and one was biopsied for the diagnosis. On histology the tiny node revealed maintained node architecture with three to four germinal centers that appeared abnormally "blue". On higher power these germinal centers were entirely composed of centrocytes and lacked tingible body macrophages [Figures 1a and 1b]. On immunohistochemistry these germinal center centrocytes expressed bcl2, CD10 and CD20 strongly [Figures 1c and 1d]. There was no interfollicular spill of these

centrocytes even on immunohistochemistry. Though the initial diagnosis in 1997 was grade I FL, it fitted the description of FLIS as described now. The peripheral blood revealed a WBC count of 32,000/cm^3 with 67% lymphocytes and 15% atypical lymphoid cells. As he was otherwise asymptomatic the patient was kept under close observation. For 11 years since diagnosis he had an uneventful follow up and never required any therapy for lymphoma. After June 2008 he has been lost to follow up.

Case 2

This 65-year-old lady presented with cervical lymphadenopathy in 2007. She had no other symptoms and a CT scan thorax and abdomen revealed no abnormality. The cervical nodes were 1.5 to 2 cm and firm. A lymph node biopsy revealed loss of architecture and typical histological features of FL grade II with neoplastic cells expressing CD20 and CD10 but were bcl2 negative. A staging bone marrow was uninvolved and as she had stage IA disease and low FLIPI (FL international prognostic index), she was kept under observation only. Three years later in 2010, she developed acute abdomen and perforative peritonitis. An emergency exploratory laparotomy was done and a segment of jejunum with the perforating mass was resected. According to the operating surgeon there were no enlarged abdominal lymph nodes but she nevertheless sampled some for histology. The jejunal mass was composed of monotonous population of intermediate-sized "blast" like lymphoid cells. On immunohistochemistry these cells were CD20, CD10 and bcl2 positive and showed a MIB1 labeling of 100%. Also noted intermixed with these cells was another component of small atypical cleaved lymphoid cells (centrocytes) with low proliferative index on MIB1 staining that marked with CD10 and CD20 but were bcl2 negative. Thus the intestinal tumor showed an intermixture of low grade bcl2 negative FL and high-grade "blastoid" transformation of FL which was bcl2 positive. A MYC translocation break apart probe (Vysis 8 q 24 LSI MYC Dual Color Break Apart Rearrangement Probe) did not reveal any abnormality. The adjacent non-tumor ileal mucosa showed hyperplastic Payer's patches with maintained architecture. The germinal centers in some follicles were clearly demarcated and were composed of mainly centrocytes, very occasional centroblasts and no tingible body macrophages and showed features of FLIS [Figure 2a-c]. FLIS involved multiple follicles in ileum and also in some part of jejunum. On immunohistochemistry the FLIS follicles showed CD20, CD10 and bcl2 positivity [Figure 2d] restricted to the germinal centers. The MIB1 index was low (5%). The regional nodes removed during surgery were uninvolved. Postoperatively the patient was advised chemotherapy but was lost to follow up.

Case 3

A 59-year-old male presented earlier this year with a right inguinal lymph node enlargement which was persistent for four years; he was otherwise asymptomatic. The biopsy showed a small lymph node with maintained architecture and there were only three scattered neoplastic follicles within the lymph node which were composed of centrocytes and centroblasts akin to FL grade III a, unlike the first two cases and showed a MIB1 labeling of 30% [Figures 3a–d]. The immunohistochemistry profile was otherwise typical with strong bcl2 and CD20/CD10 expression without involvement of the interfollicular region [Figures 3e and 3f]. This was reported as partial intrafollicular involvement by FL grade IIIa and hence patient was treated like a case of an overt FL. He had four to five other inguinal nodes on routine CT scan of 1–2 cm in size but patient refused rebiopsy. As PET CT scan revealed additionally a 5 × 4cm iliac nodal mass and a lytic lesion in iliac bone, the patient received 1 cycle of Rituximab(R) with CVP (cyclophosphamide, Oncovin (vincristine) and prednisone) chemotherapy and 5 cycles R-CHOP (cyclophosphamide, hydroxydaunorubicin Oncovin (vincristine) and prednisone). After completion of chemotherapy he received radiotherapy to the iliac lesion and abdomen. Two months after completion of all

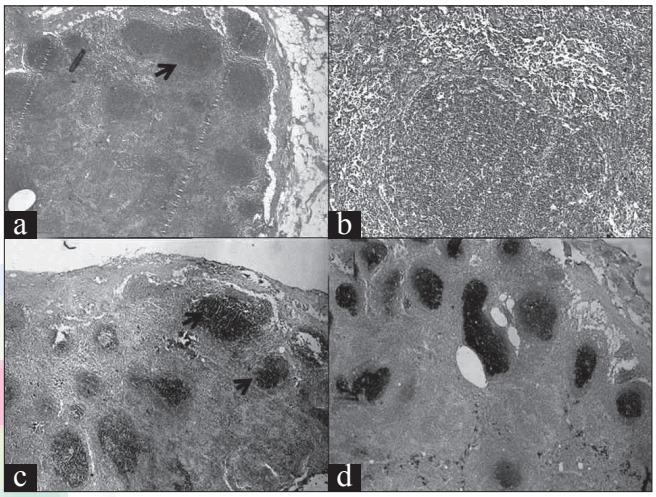

Figure 1: a) Lymph node shows few dark follicles (arrow) (H&E: ×20), b) These are composed of centrocytes only (H&E: ×200), c) Bcl2 stains these dark germinal centers (arrows) (ABC: ×20), d) CD 10 intensely stains follicles and confirms lack of germinal center cells in interfollicular region (ABC: ×20)

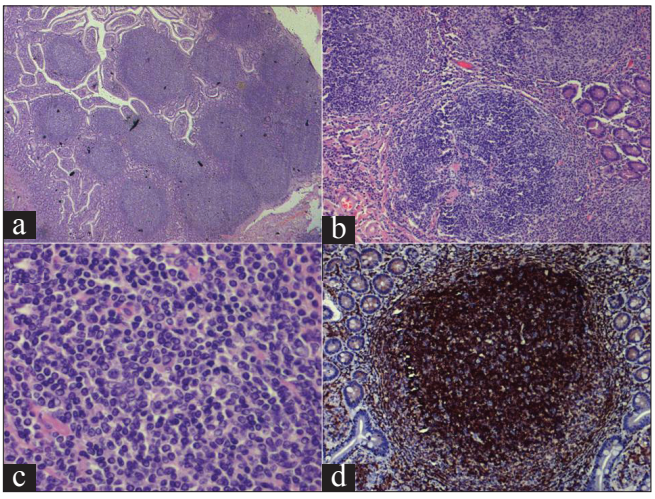

Figure 2: Case 2 shows a) Ileal mucosa with hyperplasia of Peyer's patches (H&E: ×100), b) Low power (H&E: 200) and c) High power of few germinal centers with centrocytes only (H&E: ×400) and d) intense bcl2 staining of these follicles (ABC: 200)

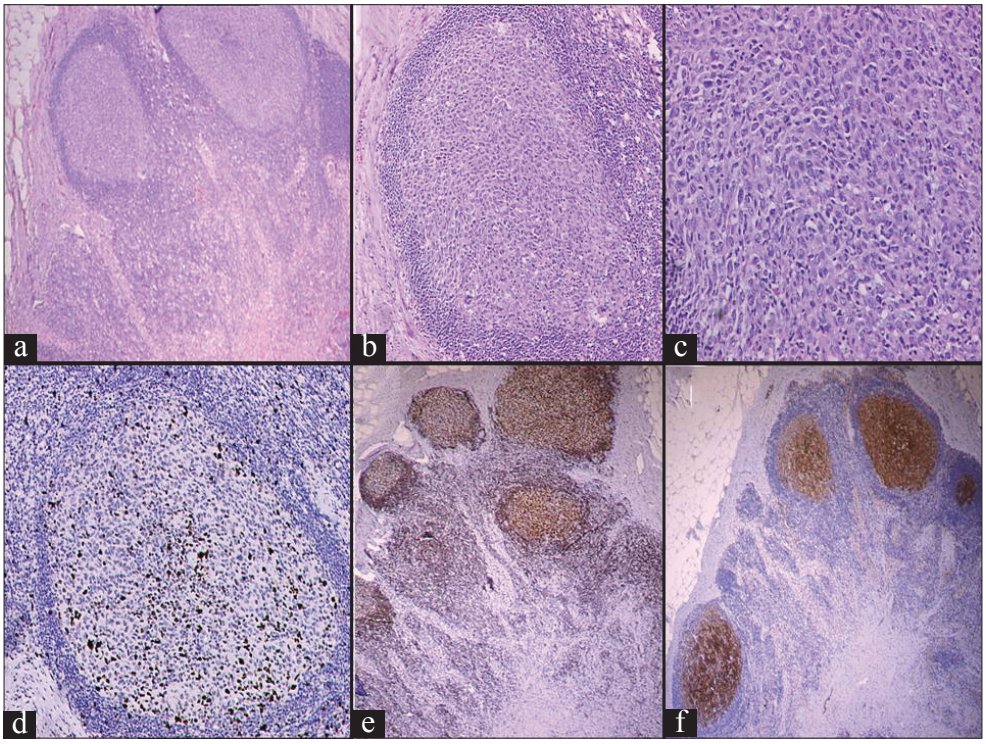

Figure 3: (a) Low power (HandE: ×20), and (b) Higher power of the lymph node shows few germinal centers with no tingible body macrophages (HandE: ×100), with (c) Admixture of centrocytes and centroblasts (HandE: ×200)., (d) Low MIB1 labeling in these germinal centers (ABC: ×200). (e) Bcl2 staining of these follicles (ABC: ×20). (f) CD10 reveals absence of interfollicular spill. (ABC:20)

chemotherapy, patient has complete response but interestingly his inguinal nodes remain enlarged at 2 cm with a SUV of 3.

DISCUSSION

FLIS was described in 2002 as being one of the early events associated in development of FL and renamed in the 2008 version of the World Health Organization lymphoma classification as 'intra-follicular neoplasia / FLIS'.[2,3] The disease is rare and the lesion was discovered in 2.5% of reactive lymph nodes examined.[2] It is characterized by the presence of germinal centers (GCs) composed of centrocytes only that strongly express Bcl-2 protein and GC markers (CD10, Bcl-6), while most of the remaining lymph node shows a pattern of reactive follicular hyperplasia, in absence of interfollicular infiltration.[2,3] The germinal centers affected by FLIS appear bluer [Figures 1 and 2] due to presence of smaller centrocytes and lack tingible body macrophages and this should prompt pathologist to ask for bcl2 staining to rule out FLIS. A dual staining for bcl2 and bcl6 is more diagnostic in ensuring that the bcl2 expression is indeed in the germinal center centrocytes.

We discuss three cases of FLIS each representing the unique clinical spectra of FLIS. Unfortunately, none of our cases had restricted single node disease and two in fact had overt disease. In essence they do not reflect the nearly benign course seen in patients with disease confined to few neoplastic germinal centers. In the latter, the mandate is to investigate the patient thoroughly and closely observe only.[1] The importance in recognition of FLIS is in the fact that a small proportion do progress to overt FL and though FL is "difficult to eradicate disease", some pathologist believe that early recognition will help understand how the disease progresses.

In case 1, patient had peripheral blood spill and marrow involvement but with an uneventful follow for over a decade with no disease progression. One of the earliest events in follicular lymphomagenesis is thought to be t(14;18)(q32;q21) translocation because of erroneous VDJ recombination in B-cell precursors developing in the bone marrow.[6] These circulating FL-like B cells with t(14;18) have been demonstrated in peripheral blood cells by highly sensitive PCR assays in as high as 23% of normal individuals.[6] These circulating t(14;18)-carrying B cells by themselves are incapable of producing a FL and require additional genetic mutations to develop into overt FL. These cells however can live longer and thus more easily accumulate additional somatic mutations developing into overt FL.[1] FLIS represents the tissue counterpart of these circulating FL-like B cells and thus actually originates in the bone marrow; hence, many pathologist feel the term intrafollicular neoplasia is a more appropriate term. FLIS associated with peripheral blood spill do not constitute an overt FL[1] and the follow up in our patient confirms the preclinical nature of such type of disease. Also in patients with multiple sites of "*in situ*" involvement, no worse prognosis has been so far documented and the wait and see policy should be adequate here too.[1]

Most patients with FLIS have an uneventful follow up. In the

three larger series of cases 13/23 (56%), 20/24 (83%) and 4/5 pure FLIS patients did not develop overt FL.[2,5,7] Because the involved nodes are tiny, FLIS is detected only when there is a coexisting pathology like metastatic carcinoma or another overt lymphoma. In a study from Spain, 5/13 patients with FLIS had another lymphoma and four had other solid tumors requiring node dissection with FLIS as an incidental finding.[7] No such association was observed in this study.

Another subgroup of FLIS occurs in association with synchronous or metachronous FL, suggesting homing to an early colonization of reactive GCs by FL,[1] and the last two cases describe this case scenario. While cases of intestinal involvement by overt FL are reported,[8,9] there is no report in literature of FLIS involving the Peyer's patch as in the case 2 we discussed. FLIS involving mucosa-associated lymphoid tissues may represent a common homing pattern of FLIS and may not be as rare. We probably picked it up because of the unusual co-existing higher grade FL. As the low-grade FL in the case 2 continued to be bcl2 negative we presume that the higher grade bcl2 positive transformation in jejunum evolved from another clone originating in the intestine as opposed to the systemic disease. Association of FLIS with bcl2 negative FL is reported. In one report of FLIS with bcl2 expression synchronously presenting with a BCL2 negative overt FL, both lesions were clonally related and carrying the t(14;18)(q32;q21) translocation.[10] This may be because in a small proportion of FL the BCL2 expression is not detected by immunohistochemistry because of mutations in the bcl2 gene and require staining with the E17 monoclonal antibody for bcl2, which detects an alternate epitope preserved in cases with these mutations. Few cases of FL may truly lack the IGH-BCL2 translocation and thus be bcl2 negative.

The case 3 in this study highlights the fact that, though technically "*In situ*" higher grade FL should be treated as they have higher incidence of overt FL elsewhere. The present studies on FLIS strictly restrict the term to centrocyte-rich lesions with low mitosis but we have included case 3 under FLIS as we are aware of similar cases being reported as intrafollicular neoplasia. Interestingly, these inguinal nodes remain enlarged in patient even though the overt disease has responded to chemotherapy. As of now all FLIS need to be staged to rule out an overt FL elsewhere but there is no data that compares higher grade FL localized to germinal center without overt disease vs. the typical FLIS.

To summarize, FLIS encompasses a nearly uniform pathologic process with many clinical presentation scenarios and while a lot had been elucidated, some angles of disease, especially management issues, in patients without overt disease are unclear.

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How to cite this article: Sakhadeo U, Mane A, Shet T. In situ follicular neoplasia/lymphoma: Three illustrative cases exemplifying unique disease presentations. Indian J Pathol Microbiol 2012;55:218-21.

Source of Support: Nil, **Conflict of Interest:** None declared.