ORIGINAL RESEARCH ARTICLE

Follicular lymphoma *in situ* in intra-abdominal lymphadenectomies—a study of five cases: revisiting the entity

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Abstract: Background: Follicular lymphoma *in situ* (FLIS) is characterized by the presence of germinal centers that strongly express BCL-2 protein and germinal center markers CD10 and BCL-6, although most of the remaining lymph node shows a pattern of follicular hyperplasia, in the absence of interfollicular infiltration. Here, we present five cases of FLIS and discuss their presentation and pathological identification in a wide variety of clinical settings. Materials and Methods: The present study includes five cases of FLIS diagnosed in the department of surgical pathology over a period of three years (2010 to 2013). The clinical data and the follow-up information were obtained from the medical records. Results: The present study included three male and two female patients with an age range of 46–72 years. One case of FLIS was associated with diffuse large B-cell lymphoma (DLBCL), while in two cases this was an incidental finding associated with other non-lymphoid malignancies. Conclusion: FLIS has a very low rate of progression to clinically significant follicular lymphoma (FL), and the management strategy recommended is to watch and wait. However, some cases may develop into full blown FL and also many non-FL lymphoid malignancies have been seen associated with it. Hence, a staging workup is strongly advocated by many authors for FLIS.

Keywords: follicular lymphoma *in situ* (FLIS); BCL-2 protein expression; clinical presentation


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Follicular lymphoma (FL) comprises approximately 20% of all lymphomas and shows presence of the t(14;18)(q32;q21) translocation in approximately 85% of cases. Normal germinal centers within lymphoid tissue lack BCL-2 and immunohistochemistry for this marker is valuable for diagnosis of FL. In 2002, follicular lymphoma *in situ* (FLIS) was defined as abnormal expression of BCL-2 confined to germinal centers and associated with preserved follicular architecture, residual reactive germinal centers and without any evidence of disseminated disease[¹,²]. Diagnosis of FLIS requires a high degree of clinical and diagnostic suspicion and it is usually diagnosed as an incidental finding. Here we present five cases of FLIS and discuss their presentation and behavior.

Materials and methods

The present study includes five cases of FLIS diagnosed in the department of surgical pathology over a period of three years (2010 to 2013) from a wide range of in-house surgical major cancer resection specimens as well as...
second opinion hematopathology consultation cases. The clinical data and the follow up information were obtained from the medical records. The excisional biopsy or the specimen of intact lymph node received was fixed in 10% formalin. The tissue was embedded in paraffin and four microns sections were examined following staining with Hematoxylin and eosin (H&E) stain. Immunohistochemical staining was performed in all five cases using the following antibodies-BCL2 (DAKO; dilution: 1:10; antigen retrieval: Ventana Ultra Benchmark Cell Conditioner 1); CD20 (DAKO; dilution: 1:200; antigen retrieval: Ventana Ultra Benchmark Cell Conditioner 1); CD10 (LEICA; dilution: 1:10; antigen retrieval: Ventana Ultra Benchmark Cell Conditioner 1); BCL-6 (VENTANA; dilution: 1:10; antigen retrieval: Ventana Ultra Benchmark Cell Conditioner 1); MIB-1 (DAKO; dilution: 1:100; antigen retrieval: Ventana Ultra Benchmark Cell Conditioner 1); CD21 (DAKO; dilution: 1:50; antigen retrieval: Enzyme protease: 8 min).

A PCR-based assay was conducted to illustrate the presence of a BCL-2 translocation in two cases. This was carried out using tissue from a formalin fixed paraffin embedded (FFPE) block. The block was sectioned and DNA was enzymatically extracted using the QIAcube instrument supplied by QIAGEN, set up according to the standard FFPE protocol. IdentiClone™ BCL2/JH Translocations Assay (Invivoscribe® Technologies) kit was used and PCR was conducted according to the manufacturer’s instructions. DNA was also checked for quality prior to set up. Post-PCR, DNA fragments were subsequently separated according to size using a polyacrylamide gel electrophoresis (PAGE) system and stained with a fluorescent dye (Gel Red-O).

Ethics statement

The authors declare no issues related to ethics with respect to the research, authorship and/or publication of this article.

Results

The present study included three male and two female patients with an age range of 46–72 years. The clinical presentation of these cases is variable and has been described as follows.

Case 1

A 72-year-old female presented to the surgical team with weight loss and small bowel obstruction. She underwent emergency small bowel resection with a clinical diagnosis of probable carcinoid tumor. Macroscopic examination revealed a large circumferential obstructive tumor in the mid-portion of small bowel along with regional mesenteric lymphadenopathy. The morphological features and the immunoprofiles (CD20, CD10, BCL-2 and BCL-6 diffusely positive) were those of a high grade diffuse large B-cell lymphoma (DLBCL)—germinal center phenotype (90%) with a minor component of FL grade three (10%) involving full thickness of the small bowel (Figure 1). One of the 12 larger mesenteric lymph nodes showed partial infiltration by the above tumor and grade 1 FL. In addition, some of the remaining small mesenteric lymph nodes showed smaller lymphoid follicles with germinal centers showing lack of zonation, tingible body macrophages and mitotic figures. There was no spillage of neoplastic lymphoid cells into the interfollicular area and these morphological features were suspicious of FLIS. A BCL-2 immunostain revealed dense expression of the same within these lymphoid follicles (Figure 1 and Figure 2). Gene rearrangement analysis demonstrated bcl2-IgH t(14;18) in the mesenteric lymph node confirming FLIS as well as in the high grade small bowel lymphoma (Figure 3). This patient was referred to a specialist center for further management. Bone marrow biopsy done was negative. She received four cycles of R-CHOP and has been in complete remission for the last 18 months.

Case 2

A 56-year-old male presented with obstructive jaundice and a pancreatic mass and after thorough investigations was diagnosed with pancreatic carcinoma. He was initially stented and later underwent a pylorus preserving pancreaticoduodenectomy. Histological examination revealed a poorly differentiated ductal adenocarcinoma of pancreas with metastasis in 3 of 18 regional lymph nodes. In addition, the cystic duct lymph node showed few enlarged follicles which showed lack of zonation and decrease in tingible body macrophages. BCL-2 staining revealed dense positive staining within these atypical follicles indicating the presence of FLIS as an incidental finding. Postoperatively he was treated with gemcitabine adjuvant chemotherapy as part of the ESPAC-4 trial. However, this was discontinued after three cycles due to side effects from gemcitabine. This patient did not have a staging bone marrow biopsy. He had a CT scan for follow up of his pancreatic malignancy and developed liver metastasis from pancreatic carcinoma 18 months after surgery. He refused chemotherapy and died two years after surgery.
Follicular lymphoma in situ in intra-abdominal lymphadenectomies—a study of five cases: revisiting the entity

Figure 1 Photomicrograph Case 1. A) Histological examination of small bowel tumor showing a high grade diffuse large B-cell lymphoma comprising sheets of large pleomorphic lymphoid cells (H&E stain 200X); B) Section from adjacent small bowel showing follicular Grade 3 component. Note the neoplastic follicle comprising centrocytes and large number of centroblasts (H&E stain 100X); C) Histological examination of the regional large mesenteric lymph node showing FL, Grade 1 comprising neoplastic follicles composed predominantly of centrocytes (H&E stain 100X); D) Section from the smaller sized mesenteric lymph nodes showing FLIS. The lymph node shows maintained follicular architecture with no expansion of interfollicular area (H&E stain 20X)

Figure 2 Photomicrograph showing comparative morphology between FLIS and low grade FL. A) FLIS: uniform-sized small lymphoid follicle with germinal center showing lack of zonation, tingible body macrophages and mitotic figures. There is no spillage of neoplastic lymphoid cells into the interfollicular area (H&E stain 100X); B) Note the dense BCL-2 immunostaining within germinal centers in FLIS (BCL-2 200X); C) FL: The lymph node shows expanded neoplastic follicles composed of centrocytes and centroblasts along with expansion of interfollicular area (H&E stain 100X); D) Note the membranous BCL-2 staining pattern in FL. The intensity of BCL-2 staining is less as compared to that seen in FLIS (BCL-2 100X)
Figure 3 PCR analysis performed on Case 2. Clear but weak bcl2-IgH gene rearrangement t(14;18) was noted in these samples. Sample 1: DLBCL and Sample 2: lymph node showing features of FLIS.

Case 3

A 53-year-old female presented with weight loss, abdominal pain and subacute small bowel obstruction. The CT scan revealed thickened terminal ileum and an obstructive tumor in the ascending colon. The patient underwent right hemicolectomy and histological examination revealed moderately differentiated adenocarcinoma of ascending colon with serosal breach and metastasis in 4 of 31 lymph nodes. In addition, the lymphoid follicles in the terminal ileum and in a few pericolic lymph nodes appeared prominent and demonstrated lack of tingible body macrophages. There was strong expression of BCL-2 and CD10 within these follicles as compared to the normal surrounding lymphoid tissue, consistent with FLIS involving pericolic lymph nodes and focally the terminal ileum. The patient received postoperative adjuvant chemotherapy for colonic carcinoma and is asymptomatic 13 months after initial surgery. Staging bone marrow biopsy was not performed as FLIS was considered to be an incidental finding.

Case 4

A 68-year-old male was presented to the surgical team with history of abdominal pain and malaise. The CT scan revealed slightly enlarged mesenteric lymph nodes. On laparotomy small bowel adhesions were noted. The appendix and two mesenteric lymph nodes were removed and reported as normal on histology. Follow-up CT scan a year later detected persistent enlarged mesenteric lymphadenitis. In view of this finding, the mesenteric lymph node biopsy was reviewed in a specialist hematology multidisciplinary team meeting, and morphological findings similar to those described in the above-mentioned cases were noted. The atypical follicles showed very strong expression of BCL-2 protein on immunohistochemistry (Figure 2). Molecular genetic studies confirmed clear, but weak bcl2-IgH gene rearrangement t(14;18) in these samples, confirming FLIS. In view of B symptoms, bone marrow biopsy was performed and this was reported to be uninvolved by lymphoma. The patient has been maintained on regular CT scan follow-up with a wait-and-watch policy and the CT scan at the end of 20 months revealed slight increase in the size of the mesenteric lymph nodes, but with no palpable peripheral lymph nodes and hepatosplenomegaly. A repeat biopsy was performed in view of the slight increase in size, but demonstrated only dense perinodal fibrosis and T-zone paracortical expansion. There was no evidence of any malignancy. It was very difficult to ascertain whether the symptoms were initially due to the lymphadenopathy containing FLIS or due to small bowel adhesions. Lymph node biopsies performed three times over a period of three years did not reveal any evidence of full-blown lymphoma on biopsy.

Case 5

A 46-year-old female presented malaise and vague abdominal pain. The CT scan examination revealed low volume minimal mesenteric lymphadenopathy. Laparoscopic mesenteric lymph node biopsy was performed. Histologically these were two small lymph nodes, 3 mm each that showed atypical lymphoid follicles with dense BCL-2 staining within germinal centers, in keeping with FLIS. On the post biopsy PET scan, a 25 mm nodal mass was still identified in the small bowel mesentry that was PET positive. The case was referred to the tertiary specialist hematology center for second opinion. The lymph node biopsy was reviewed at the center and there was definite FLIS. However, further levels showed presence of occasional small cluster of strong BCL-2 and CD20 positive B-cells in the interfollicular area and in the perinodal fat. Following this review at the specialist center, the histology was reported as FLIS with a minor component of grade 1 FL. In view of the presence of PET positive lymph node mass, the patient was treated with “involved site radiotherapy” to a dose of 24 Gy in 12 fractions. The post-treatment CT scan revealed an excellent response and the patient is now in remission six months post treatment.

Discussion

The diagnosis of FLIS requires a high degree of clinical
and diagnostic suspicion as they are often incidental findings in otherwise reactive appearing lymph nodes[3]. Its prevalence reported in literature is 2.3%[4]. FLIS is characterized by the presence of widely scattered germinal centers that strongly express BCL-2 protein and germinal center markers CD10 and BCL-6, while most of the remaining lymph node shows a pattern of follicular hyperplasia, in the absence of interfollicular infiltration. This should be distinguished from partial involvement by follicular lymphoma (PFL), where the involved follicles are expanded, grouped together, show variable staining intensity for BCL-2 and show atypical cells that are very low rate of progression to clinically significant which FLIS can be encountered. As such, FLIS has a

Follicular lymphoma in situ (FLIS) is highly recommended for the possible coexistence of an overt lymphoma. Park et al. describes a case of FLIS that developed after Hodgkin lymphoma (HL). During follow-up, the PET/CT scan revealed increased FDG uptake in cervical and axillary lymph nodes that suggested recurrence of HL. However, the biopsy revealed small lymph nodes with features of FLIS, but no HL[9]. Similar findings were noted in the fifth case of our series where-in the PET scan showed increased FDG uptake; however the biopsy revealed only small nodes with FLIS and a minor component of grade 1 FL. One should be aware that increased FDG uptake can be seen in FLIS and hence these findings should be interpreted with caution in the right clinical setting. Case 5 is a very typical example of the term “microinvasion” commonly used in cervix stage pT1a1 lesions, where we use the term CIN3 (squamous carcinoma in situ) with microinvasion and melanocytic lesions, where we use melanocytic intraepidermal neoplasia (MIN) with microinvasion. Such terms, however, are not used in haematopathology.

In case 5, the lymph node showed predominantly FLIS with spillover of neoplastic B-cells in the interfollicular area and perinodal fat, indicating a minor component of grade 1 FL.

There is no evidence for starting any therapy for multifocal ‘in situ’ lymphoma, and the wait-and-watch policy is strongly suggested. Follow-up with imaging has been advocated only in the presence of disease-related symptoms or organ involvement[10]. Two of our cases, who had incidental FLIS did not show any evidence of FL following staging procedures.

The third case of our series had a non-lymphoid malignancy of the ascending colon, but also demonstrated features of FLIS in the terminal ileum and some pericolonic lymph nodes. The patient did not have any imaging or staging bone marrow biopsy but is asymptomatic 13 months after initial surgery. We believe that this might represent colonization of mucosa associated lymphoid tissue by circulating t(14;18) carrying B-cells that are found in 23% of normal individuals, but they are incapable of producing a FL due to lack of additional somatic mutations. Similarly, Carbone et al. described two cases: one case of FLIS with prostatic carcinoma and the other case of FLIS occurring six months after the resection of carotid body parangangioma[11]. Cases associated with lung[12], colon[13] and breast adenocarcinoma[14] have
also been described in literature. These cases raise the issue of prognostic significance of FLIS in this clinical context. Certain open questions still need to be answered, especially regarding the association of FLIS with non-lymphoid malignancies, whether they are mere incidental, related to previous treatment or to immunosuppression. Also, the question of how should one approach such cases with a certain risk of progression to overt lymphoma remain. Recently, Morita K et al. concluded in their study that a longer period of follow-up studies is necessary to assess progression to overt lymphoma and the rate of BCL-2 positive follicular proliferation could be the factor to predicting development of FL in prospective studies. Further studies still warrant the address of these issues.

This is a unique study involving cases of intra-abdominal lymphadenectomies. The study was undertaken with a view to address the diagnostic difficulties usually encountered in these specimens, especially when these are dealt with by general or gastrointestinal pathologists and not by hematopathologists. In this era of sub-specialization when all hematology cases are dealt with or will be dealt with by specialist hematology centers, cases of FLIS are likely to be overlooked due to lack of awareness when they are associated with other non-lymphoid cancers and dealt with by general pathologists. Hence, if more such cases are reported in literature, then the awareness of such entities will be improved. Finally, in summary, FLIS is a distinct entity with an indolent clinical course first described in 2002. However, awareness about this entity is limited. They are often incidental findings in otherwise normal appearing lymph nodes. As described in previous studies, they have a very low risk of progression to FL and many non-FL lymphoid malignancies have been associated with it. A staging workup is recommended by many authors and a watch-and-wait management strategy is advised.

**Conflict of interest**

The authors declared no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

**References**