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In Situ Follicular Lymphoma Developed after Hodgkin Lymphoma

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In situ follicular lymphoma is a newly defined entity among the lymphoid neoplasms and is defined as architecturally normal-appearing lymph nodes and other lymphoid tissues that have one or more follicles that demonstrate bcl-2 overexpressing centrocytes and centroblasts, with or without a monomorphic cytologic appearance suggestive of follicular lymphoma. Here we present a case of *in situ* follicular lymphoma diagnosed during the follow-up after a complete response to the treatment of lymphocyte-rich classical Hodgkin's lymphoma. In our case, because only a few germinal centers contained bcl-2 overexpressing cells, we missed the diagnosis of *in situ* follicular lymphoma in the initial histological examination. We could establish the diagnosis only after performing bcl-2 immunostaining in the sequential biopsy. Therefore, we recommend that careful histological examination along with bcl-2 immunostaining is needed in patients with suspicious clinical findings.

Key Words: Lymphoma; Bcl-2 protein; Preneoplastic conditions

Intrafollicular neoplasia/*in situ* follicular lymphoma is a newly defined pathologic entity included in the 4th edition of the World Health Organization (WHO) classification of the lymphoid neoplasms.[1] New WHO classification defined the intrafollicular neoplasia/*in situ* follicular lymphoma as architecturally normal-appearing lymph nodes and other lymphoid tissues that have one or more follicles that demonstrate bcl-2 overexpressing centrocytes and centroblasts, with or without a monomorphic cytologic appearance suggestive of follicular lymphoma.[1] Morphologically, *in situ* follicular lymphoma closely resembles the normal germinal center of secondary lymphoid follicles. Therefore, *in situ* follicular lymphoma is difficult to recognize without bcl-2 staining.[1-3] Hence, an awareness of this entity in the pathologic diagnosis of lymph node biopsy specimens is needed. Here, we present a recently experienced case of *in situ* follicular lymphoma which was overlooked during the first follow-up biopsy and was diagnosed in the sequential follow-up biopsy after a complete response to the treatment of lymphocyte-rich classical Hodgkin's lymphoma.

CASE REPORT

A 65-year-old female patient was being followed up after a complete response to the lymphocyte-rich classical Hodgkin's lymphoma diagnosed three years ago (Fig. 1A). The patient had no specific symptoms such as the B symptoms. However, follow-up positron emission tomography/computed tomography (PET/CT) images showed increased FDG uptake in both the cervical and axillary lymph nodes which suggested recurrence of Hodgkin's lymphoma (Fig. 1B). For the confirmatory diagnosis, excision of the axillary lymph nodes was performed. His-

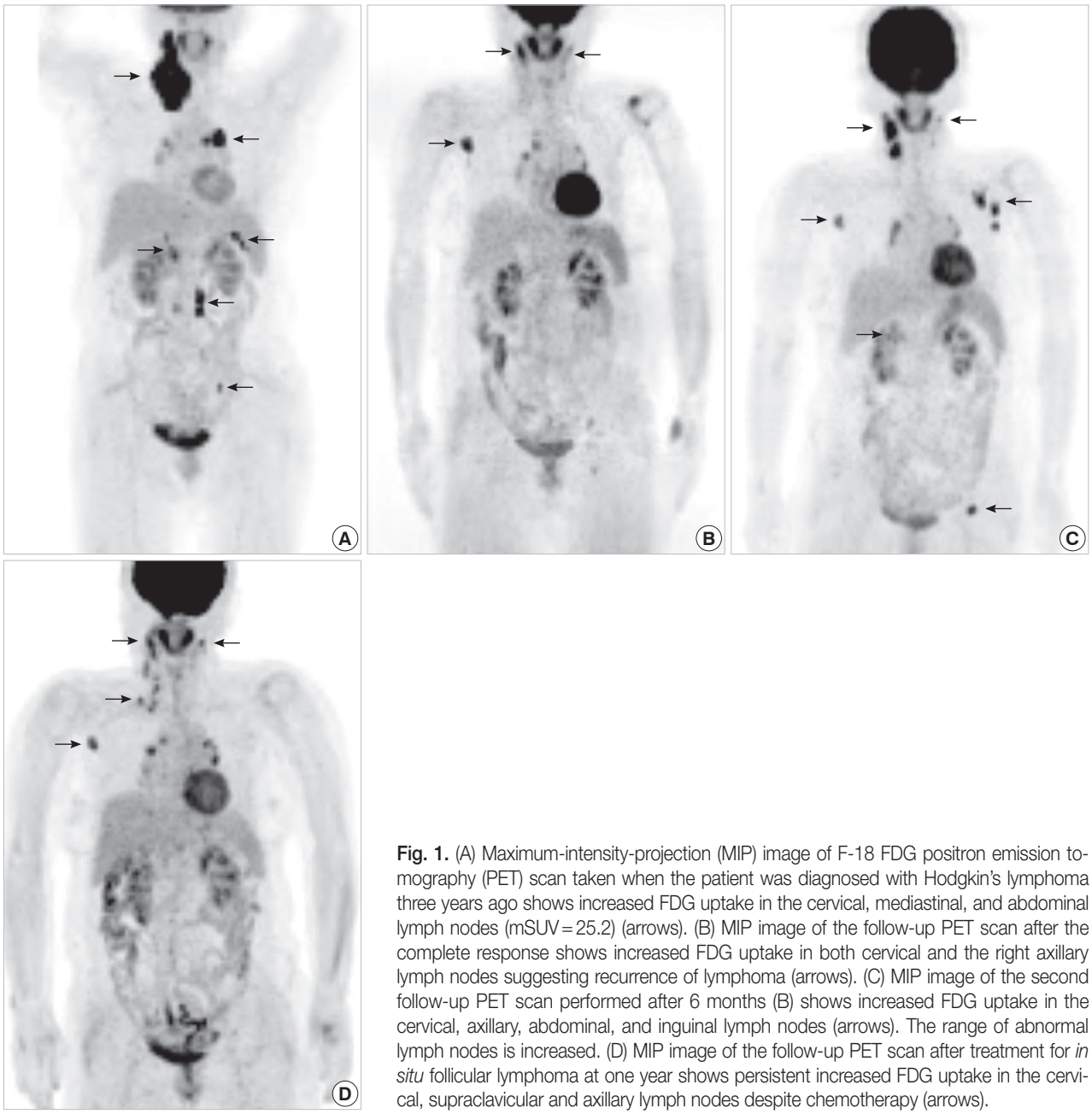

Fig. 1. (A) Maximum-intensity-projection (MIP) image of F-18 FDG positron emission tomography (PET) scan taken when the patient was diagnosed with Hodgkin's lymphoma three years ago shows increased FDG uptake in the cervical, mediastinal, and abdominal lymph nodes (mSUV = 25.2) (arrows). (B) MIP image of the follow-up PET scan after the complete response shows increased FDG uptake in both cervical and the right axillary lymph nodes suggesting recurrence of lymphoma (arrows). (C) MIP image of the second follow-up PET scan performed after 6 months (B) shows increased FDG uptake in the cervical, axillary, abdominal, and inguinal lymph nodes (arrows). The range of abnormal lymph nodes is increased. (D) MIP image of the follow-up PET scan after treatment for *in situ* follicular lymphoma at one year shows persistent increased FDG uptake in the cervical, supraclavicular and axillary lymph nodes despite chemotherapy (arrows).

tologically, an intact lymph node capsule and follicular hyperplasia were seen. The most germinal centers of the lymphoid follicles showed tingible body macrophages and an intermixed population of centrocytes and centroblasts (Fig. 2A). Therefore, the diagnosis of reactive lymphoid hyperplasia was made. However, the second follow-up PET/CT which was performed after six months showed increased FDG uptake in the cervical, axillary, intra-abdominal, and inguinal lymph nodes (Fig. 1C). The range of abnormal lymph nodes had increased. Therefore, recurrence of Hodgkin's lymphoma was strongly suspected. Fine needle aspiration of cervical lymph nodes was performed. Cytologic smear slides showed small lymphoid cells. Small lymphocytes appeared like mature lymphocytes and centrocyte-like cells were predominant. Reed-Sternberg cells were not identified and tingible body macrophages were very rarely identified. Cytological findings were suggestive of reactive lymphoid hyperplasia. However, because the clinical findings suggested that the disease seemed to progress, diagnostic excision biopsy was recommended. On operation, despite the high FDG uptake in lymph nodes on PET/CT, the size of the cervical lymph nodes

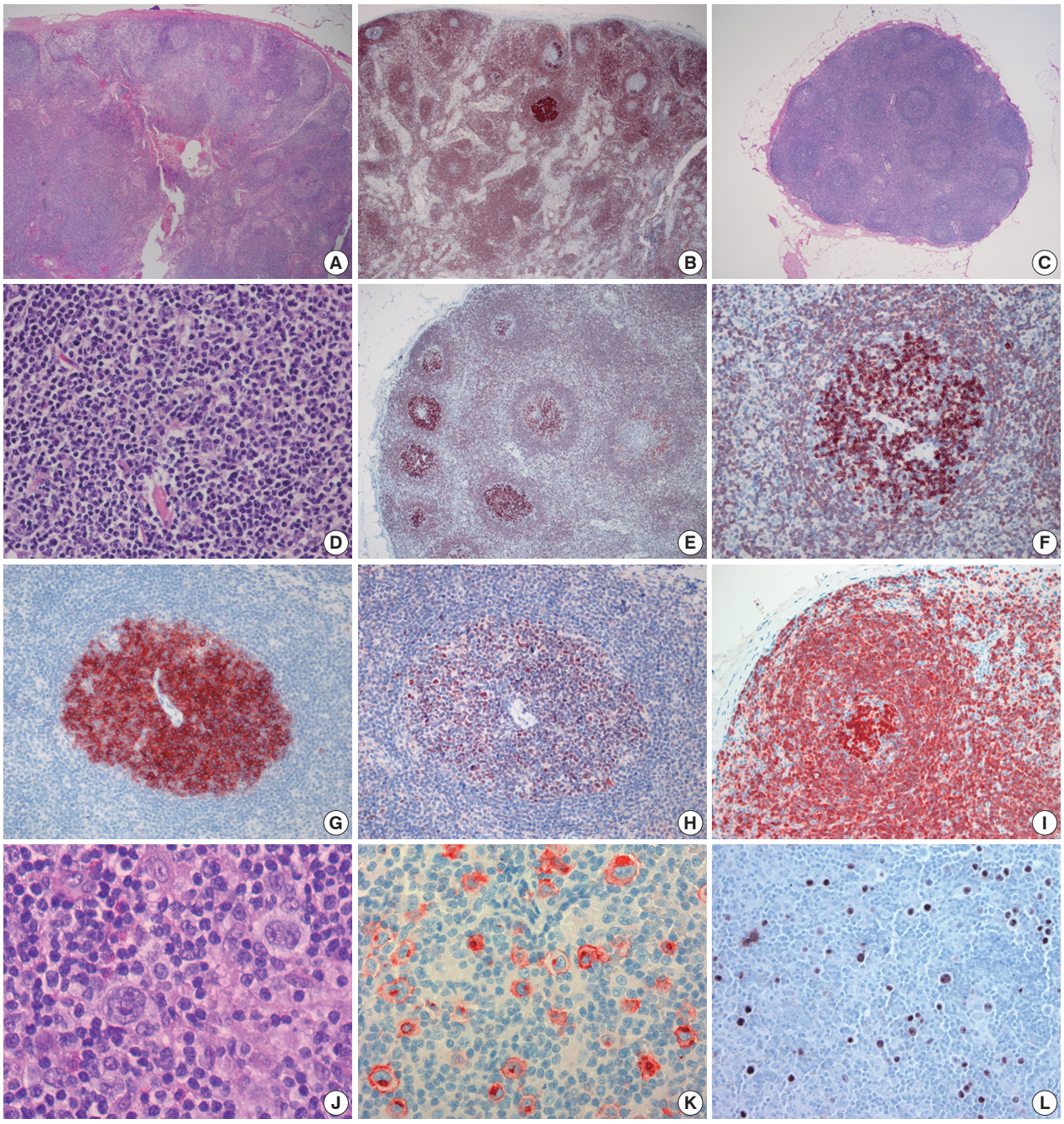

Fig. 2. (A) First biopsy specimen showing the lymph node with several reactive-appearing follicles. (B) However, focal germinal center cells stain strongly positive for bcl-2. (C) Second biopsy specimen showing many lymphoid follicles at low-power view. (D) High-power view showing a follicle with its germinal center involved by follicular lymphoma cells with predominance of small centrocytes and few centroblasts. (E, F) A bcl-2 stain reveals a variable number of germinal center cells strongly positive for bcl-2, with some germinal centers containing only a few bcl-2 positive cells. (G, H) Germinal center cells that show strong positivity for bcl-2 are also positive for CD10 (G) and bcl-6 (H). (I) Third biopsy specimen shows germinal center cells that are strongly positive for bcl-2. (J) Reed-Sternberg Hodgkin cells are intermixed with lymphocytes, histiocytes, and eosinophils. (K, L) Reed-Sternberg Hodgkin cells are positive for CD30 (K) and *in situ* hybridization for Epstein-Barr virus-encoded small RNA (L).

was very small. One small lymph node measuring 0.6×0.5 cm was excised. Histologically, the lymph node capsule was intact and the lymph node sinuses were also well preserved. Many lymphoid follicles with well-formed germinal centers were iden-

tified in the lymph node (Fig. 2C). The mantle zone was also well preserved. The germinal centers were composed of centrocytes and a few centroblasts. Mitosis and tingible body macrophages were rarely identified in most germinal centers (Fig. 2D). Histological features of lymph nodes corresponded to follicular hyperplasia. However, new entities such as *in situ* follicular lymphoma and *in situ* mantle cell lymphoma, described in the 4th edition of the WHO classification of the lymphoid neoplasms, could not be diagnosed without immunohistochemical staining. Therefore, to rule out these two new entities, immunohistochemical staining was performed. Immunohistochemically, some germinal center cells overexpressed bcl-2 and other germinal center cells either partly expressed or did not express bcl-2 in both the first and second axillary lymph node biopsy specimens (Fig. 2E, F). Germinal center cells were also positive for CD10 and bcl-6 (Fig. 2G, H). Cells expressing CD30 or cyclin-D1 were not identified. According to the diagnostic criteria of the WHO classification of the lymphoid neoplasms,[1] we diagnosed this case as *in situ* follicular lymphoma. We also retrospectively reexamined the previously excised axillary lymph node specimen based on which the diagnosis of reactive lymphoid hyperplasia was made. Interestingly, a few lymphoid follicles had bcl-2 overexpressing germinal center cells (Fig. 2B). Histologically, the bcl-2 overexpressing germinal center cells were predominantly composed of centrocytes and a few centroblasts. Moreover, in contrast to the reactive non-neoplastic germinal centers, tingible body macrophages and mitosis were rarely identified. Therefore, we re-diagnosed this case as *in situ* follicular lymphoma based on the results of the axillary lymph node biopsy specimen. The clinical findings suggested that the disease was progressing. In addition, because there was an increased number of bcl-2 overexpressing germinal center cells in the newly excised cervical lymph node biopsy specimen compared with those in the previous axillary lymph node biopsy specimen performed six months ago, we decided to treat the patient according to the conventional treatment option for follicular lymphoma. The patient was treated with six cycles of cyclophosphamide, vincristine and prednisolone (CVP) regimen. During the one year follow-up period, follow-up PET/CT showed multiple residual hypermetabolic lymph nodes in the cervical, supraclavicular and axillary areas despite chemotherapy (Fig. 1D). Sixteen months after the excisional biopsy of cervical lymph nodes, the patient underwent excisional biopsy of other cervical lymph nodes because the cervical lymph nodes showed an increase in size and uptake on follow-up PET/CT. On histological and immunohistochemical study, *in situ* follicular lymphoma was still detectable in the cortical area of the lymph nodes (Fig. 2I). Moreover, recurrent classical Hodgkin's lymphoma also coexisted in the medullary region of the lymph nodes. Reed-Sternberg Hodgkin cells were identified and were intermixed with lymphocytes, histiocytes, and a few eosinophils (Fig. 2J). Reed-Sternberg Hodgkin cells were positive for CD30 immunostaining and *in situ* hybridization for Epstein-Barr virus-encoded small RNA (Fig. 2K, L). Reed-Sternberg Hodgkin cells were negative for CD3, CD20, CD15, and CD56. Because of the recurrence of Hodgkin's lymphoma, the patient was treated with ifosfamide, carboplatin and etoposide regimen and had regular follow-up.

DISCUSSION

In situ follicular lymphoma was described as '*in situ* localization of follicular lymphoma' in 2002.[4] The reason for using the term '*in situ* localization of follicular lymphoma' was that only a very few follicles within the lymph node were involved, and the architectural and cytological features were otherwise benign.[4] Thereafter, it was renamed in the 2008 version of the WHO classification of the lymphoid neoplasms as 'intrafollicular neoplasia/*in situ* follicular lymphoma'.[1] However, the significance of this entity is currently unclear. Moreover, as defined in the WHO classification of lymphoid neoplasms, it is impossible to diagnose *in situ* follicular lymphoma based only on histologic findings without the support of bcl-2 immunostaining.[1] In our case, the patient was being followed up for a previous Hodgkin's lymphoma and the clinical findings seemed to indicate that the disease in the lymph nodes had progressed. Therefore, despite the previous biopsy based on which the patient was diagnosed with reactive lymphoid hyperplasia, a sequential biopsy was performed. Although the lymph nodes showed high FDG uptake in the PET/CT, the size of the excised lymph node was very small. It was only 0.6 cm in size and the overall architecture of the lymph node was well preserved at a low-power view. The only finding that suggested the possibility of neoplasm was that the germinal center cells had a relatively homogeneous appearance and tingible body macrophages and mitosis were rarely identified. Although, follicular lymphoma following Hodgkin's lymphoma is unusual, an additional immunohistochemical staining was performed and this case was diagnosed as *in situ* follicular lymphoma.

The clinical significance of *in situ* follicular lymphoma is that some patients are found to have follicular lymphoma elsewhere,

either before or simultaneously, while some develop overt follicular lymphoma later, and no evidence of follicular lymphoma is seen in the others.[3-5] Cong *et al.*[4] have shown that *in situ* follicular lymphoma with many bcl-2 overexpressing germinal center cells tends to be associated with overt follicular lymphoma. However, there was no definitive consensus for the treatment of *in situ* follicular lymphoma. If overt follicular lymphoma is recognized by careful evaluation of other disease sites, adequate further therapy will be required. However, in the case in which the clinical and pathologic evaluations are negative, a conservative approach without further therapy has been suggested.[4] In our case, the findings of the sequential biopsy and follow-up PET/CT suggested that the disease was progressing. Therefore, chemotherapy was started. However, a complete response was not achieved. Multiple abnormal lymph nodes did not disappear despite the CVP chemotherapy regimen. On the other hand, when we stopped chemotherapy despite the patient not achieving a complete response, the lesion progressed slowly. Moreover, Hodgkin's lymphoma recurred. In order to establish a clinical guideline for *in situ* follicular lymphoma such as its treatment modality or clinical outcome, more long-term follow-ups of many such cases are needed.

Another interesting finding in our case was that the *in situ* follicular lymphoma developed after Hodgkin's lymphoma. Coexistence of Hodgkin's lymphoma and non-Hodgkin's lymphoma or sequential development of non-Hodgkin's lymphoma in Hodgkin's lymphoma patients has been observed occassionally. The most common type of non-Hodgkin's lymphoma developing with Hodgkin's lymphoma was follicular lymphoma. It has been suggested that the classical Hodgkin's lymphoma and the follicular lymphoma originate from the same germinal center B cells.[6] However, in our case, it is not clear whether or not these two types of tumors are related. Recently, Montes-Moreno *et al.*[5] have reported five cases of *in situ* follicular lymphoma associated with lymphoproliferative disorders other than follicular lymphoma. Among them, two cases were associated with the classical Hodgkin's lymphoma. Other three cases included two cases of splenic marginal zone lymphoma and one case of diffuse large B-cell lymphoma.[5] Additionally, among the 25 cases of *in situ* follicular lymphoma, two cases of *in situ* follicular lymphoma associated with another low-grade B-cell lymphoma were identified.[4]

In conclusion, although the incidence of *in situ* follicular lymphoma is very low, awareness of the early neoplastic lesions of the lymphoid tissue is needed. This is especially true in cases where clinical findings are progressive and suggestive of lymphoma. Because *in situ* follicular lymphoma is not easily recognizable, careful histological examination of all lymphoid follicles is recommended, whether or not it is mainly composed of centrocytes or tingible body macrophages and mitosis is seen. Thereafter, an additional immunohistochemical staining for bcl-2 will be very helpful for the identification of *in situ* follicular lymphoma.

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