Case Study

Systemic Follicular Lymphoma with Massive Intestinal Involvement with Leukemic Manifestation

Yuichiro Ono,[1] Kazunari Aoki,[1] Aiko Kato,[1] Hiroshi Arima,[1] Yohko Takiuchi,[1] Seiji Nagano,[1] Sumie Tabata,[1] Sohshi Yanagita,[1] Akiko Matsushita,[1] Hayato Maruoka,[2] Yukihiro Imai,[3] Takayuki Ishikawa,[1] and Takayuki Takahashi[1]

A 30-year-old man was referred to our hospital with leukocytosis and fecal occult blood. His white blood cell count was 30.2×10^9/L with 79% small- to medium-sized lymphocytes. Surface antigen analysis revealed that these lymphocytes were positive for CD19, CD20, CD10, and CD23, but negative for CD5. The lymphocytes infiltrated the bone marrow. On endoscopic examination of the duodenum and jejunum, many small polypoid lesions were observed. A histologic picture of a biopsied lesion showed diffuse infiltration of small- to medium-sized lymphocytes in the submucosal region. On immunohisto-chemistry, these lymphocytes were positive for CD20, BCL2, and CD10 (weakly). Polymerase chain reaction analysis of cells from peripheral blood, bone marrow, and intestinal lesion showed a fusion product of *BCL2* and *immunoglobulin heavy chain* (*IGH*) genes. The fused *BCL2/IGH* gene was also demonstrated by fluorescence *in situ* hybridization in the same cell sources. Computed tomography scanning showed marked wall thickening throughout the small intestine and enlarged mesenteric lymph nodes. A diagnosis of follicular lymphoma with massive intestinal involvement in a leukemic state was made. After 6 courses of rituximab-combined CHOP chemotherapy, complete remission was obtained. 〔*J Clin Exp Hematopathol 51*(*2*) : *135-140, 2011*〕

Keywords: primary intestinal follicular lymphoma, blood involvement, leukemic lymphomatosis

INTRODUCTION

Nodal follicular lymphoma (FL) is a hematologic malignancy representative of indolent lymphoma. It frequently involves lymph nodes, but also bone marrow and peripheral blood. On the other hand, primary intestinal follicular lymphoma (PIFL) is a provisional clinical entity newly added to the World Health Organization (WHO) Classification of Hematologic Malignancies in 2008.[1] Although conventional nodal FL and PIFL carry the same chromosomal abnormality of t(14;18), PIFL almost exclusively affects duodenum and small intestine and rarely infiltrates other organs or tissues.[2] Therefore, PIFL mostly stays in the I to II, but not III to IV, clinical stages.[2] We here report a characteristic case of leukemic FL with massive intestinal involvement at presentation.

CASE REPORT

A 30-year-old man was referred to our hospital with leukocytosis and fecal occult blood, although he was asymptomatic. His past medical history was unremarkable. Physically, neither superficial lymphadenopathy nor hepatosplenomegaly was noted. His white blood cell (WBC) count was 295×10^9/L with 68% small- to medium-sized lymphocytes. Surface antigen analysis with flow cytometry revealed that these lymphocytes were positive for CD19 (100%), CD20 (99%), CD10 (99%), and CD23 (35%), but negative for CD5 (0%). On endoscopic examination, many whitish polypoid lesions were observed from the superior flexure to the descending part of the duodenum; therefore, he was admitted for further examination.

On admission, a hematologic examination showed a WBC count of 30.2×10^9/L with 79% similar lymphocytes with slightly cleaved nuclei, a hemoglobin concentration of 14.1 g/dL, and a platelet count of 365×10^9/L. On biochemical and serologic examinations, serum concentrations of aspartate

aminotransferase, lactate dehydrogenase, alanine aminotransferase, alkaline phosphatase, and γ-glutamyltransferase were 22 IU/L, 14 IU/L, 172 IU/L, 147 IU/L, and 13 IU/L, respectively, being all within normal limits. Serum levels of blood urea nitrogen, creatinine, C-reactive protein, and creatine phosphokinase were all non-specific. The serum concentration of immunoglobulin G (IgG) was low at 498 mg/dL (normally 870 to 1,700 mg/dL), while those of IgA and IgM were within the normal ranges. The serum level of soluble interleukin-2 receptor was markedly elevated to 1,929 IU/L (normally 150 to 505 IU/L). A bone marrow aspirate showed a nucleated cell count of 23.1×10^4/mL with 36.4% granuloid cells, 16% erythroid cells, and 10.4% abnormal lymphocytes similar to those seen in the peripheral blood (Fig. 1). On flow cytometry, these marrow lymphocytes were positive for CD19 (100%), CD20 (96%), CD10 (97%), and CD23 (25%).

Abdominal ultrasonography showed mild splenomegaly and enlarged mesenteric lymph nodes. Computed tomography (CT) scanning demonstrated marked wall thickening throughout the small intestine and many enlarged mesenteric lymph nodes (Fig. 2). Hepatosplenomegaly was not evident because the sizes of the liver and spleen were within standard ranges on CT imaging.

Endoscopic examination of the small intestine using a double-balloon endoscope showed many whitish polypoid lesions from the superior duodenal flexure to the jejunum (Fig. 3). The esophagus and stomach were intact on the endoscopy. Colonoscopy was not performed because CT imaging did not show significant wall thickening of the colon. A histologic image of a needle-biopsied lesion showed diffuse infiltration of small- to medium-sized lymphocytes in the submucosal region. On immunohistochemistry, these lymphocytes were positive for CD19, CD20, and BCL2 (Fig. 4), but negative for CD5 and cyclin D1. CD21 immunostaining showed loosely clustered follicular dendritic cells (FDCs) (Fig. 4). Flow cytometric analysis of cells from the biopsy specimen showed that 93% and 92% of CD19-positive cells were positive for CD10 and CD20, respectively. Polymerase chain reaction (PCR) analysis of circulating lymphocytes showed a fusion product of *BCL2* and *immunoglobulin heavy chain* (*IGH*) genes. Long-distance PCR technique using MBR/01 and Eμ primers (Table 1) demonstrated that the
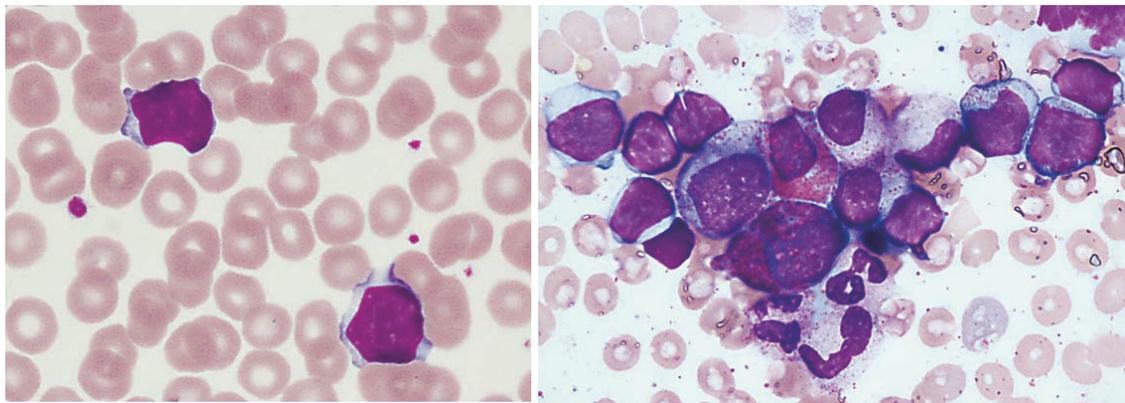

Fig. 1. Abnormal lymphocytes in the peripheral blood (*left*) and bone marrow (*right*). These lymphocytes are mature and small- to medium-sized with slightly cleaved nuclei and scant cytoplasm.

Table 1. Sequences of the primers to obtain a polymerase chain reaction product spanning the fusion portion of *IGH/BCL2*[4]

Primer designation	5' to 3' sequences	Specificity (strand/orientation)
BCL2		
MBR/01	CACAAGTGAAGTCAACATGCCTGCCCCAAACAAAT	*BCL2* exon 3, coding region (S/F)
mcr/01	GGTAGAGGTGAATACCCCAGGGCTGAGCAGGAAGG	*BCL2*, 10 kb upstream of mcr (S/F)
mcr/02	TGTTGGTTGACATTTGATGGCTTTGCTGAGAGGTA	*BCL2*, mcr (S/F)
IGH		
Eμ/01	CTAGGCCAGTCCTGCTGACGCCGCATCGGTGATTC	Enhancer region of *IGH* (A/R)

S, sense strand ; A, antisense strand ; F, forward direction ; R, reverse direction ; MBR, major breakpoint region ; IGH, immunoglobulin heavy chain gene ; E, enhancer.
Modified from Akasaka T *et al.*[4]
(MBR/01 primer is a 35-mer oligonucleotide primer for the coding region of BCL 2 exon 3, which is 2.2 kb upstream of the MBR. Eμ/01 is a primer for enhancer region of IGH, which is located 1.7 kb downstream of JH6 segment.)
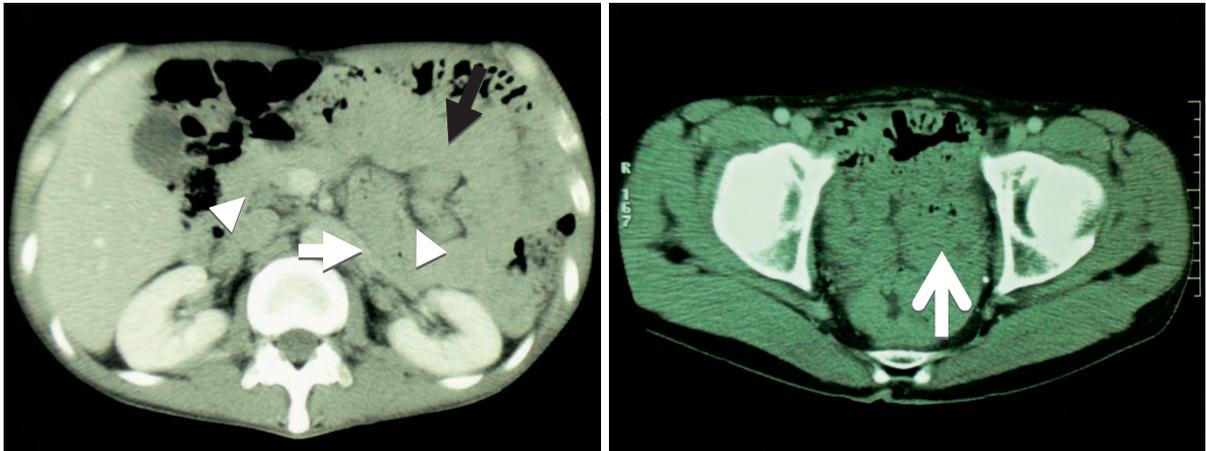
Fig. 2. Computed tomography scanning of the abdomen. Marked wall thickening of the whole small intestine (*arrows*) and many enlarged mesenteric lymph nodes (*arrowheads*) are seen.
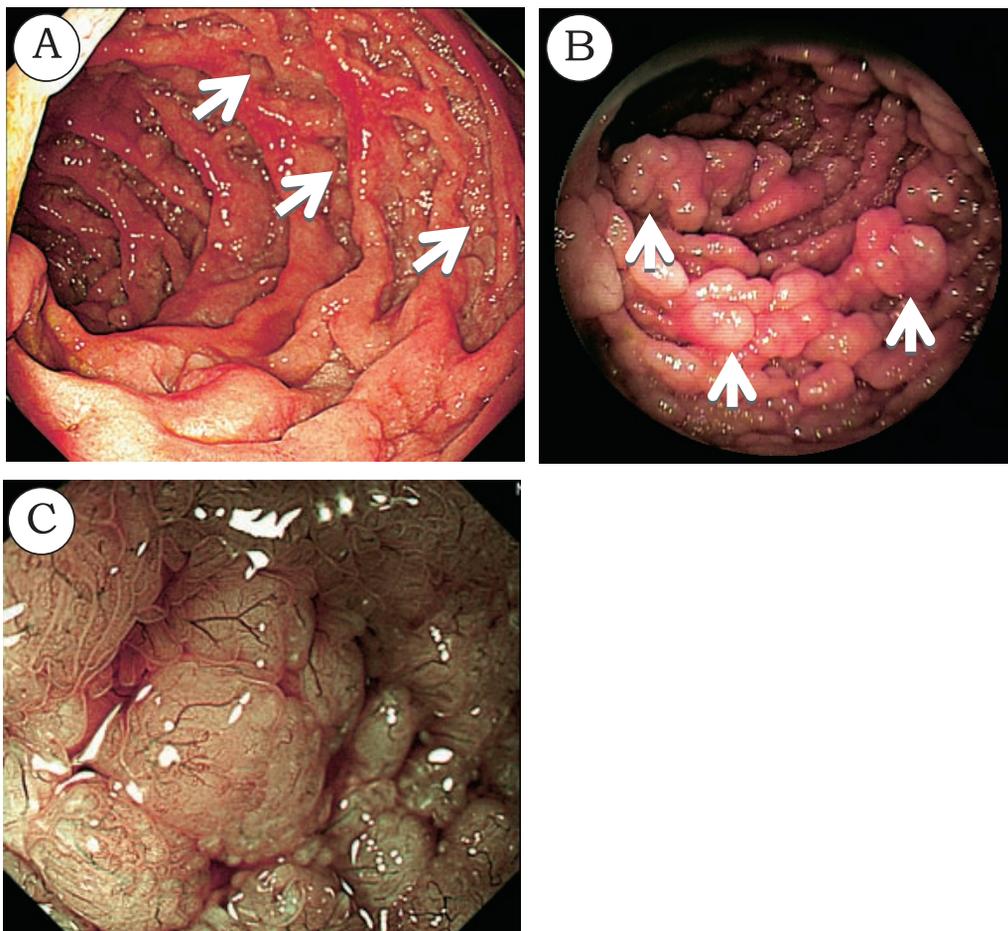

Fig. 3. Endoscopic examination of the duodenum (*3A*) and small intestine (*3B*) using a double-balloon endoscope. Many whitish polypoid lesions are seen. (*3C*) Magnified configuration of a polypoid lesion in the small intestine.
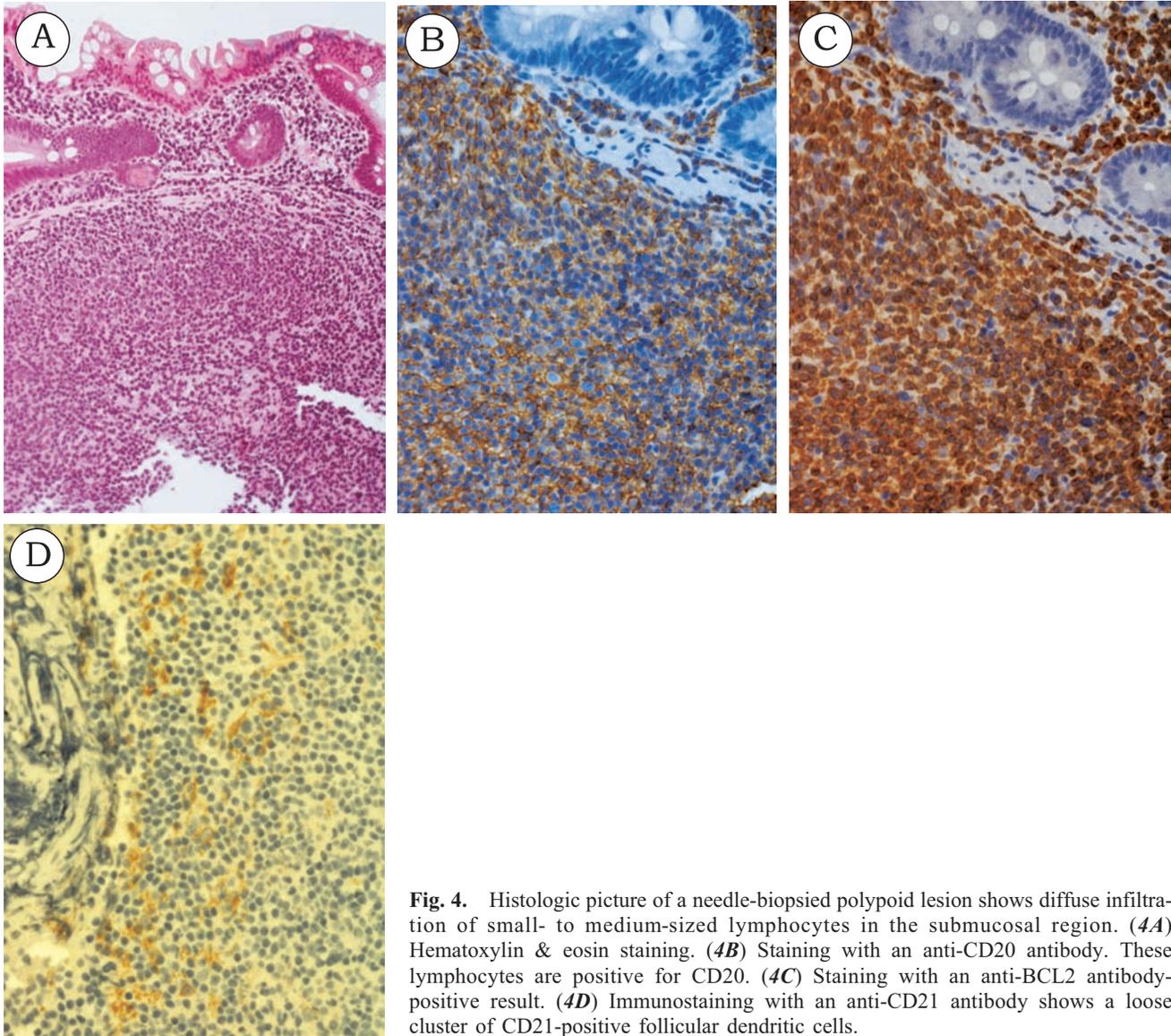
Fig. 4. Histologic picture of a needle-biopsied polypoid lesion shows diffuse infiltration of small- to medium-sized lymphocytes in the submucosal region. (**4A**) Hematoxylin & eosin staining. (**4B**) Staining with an anti-CD20 antibody. These lymphocytes are positive for CD20. (**4C**) Staining with an anti-BCL2 antibody-positive result. (**4D**) Immunostaining with an anti-CD21 antibody shows a loose cluster of CD21-positive follicular dendritic cells.

BCL2/IGH rearrangement occurred at a major breakpoint region (MBR) that can be observed in conventional nodal follicular lymphoma.[3,4] Fluorescent *in situ* hybridization analysis showed the same fusion gene in the specimens from both peripheral blood and bone marrow. Karyotypic analysis of both cell sources cultured for 24 hr in the presence or absence of the mitogen failed to show dividing cells. Gallium scintigraphy showed abnormal accumulation in the duodenum, small intestine, and mesenteric lymph nodes (Fig. 5). From these findings, the patient was diagnosed with PIFL, Grade 1, clinical stage IV, FL international prognostic index score 1 ("Low"), according to the WHO Classification 2008, Lewin's criteria,[5] International Workshop criteria,[6] and Solal-Celigny *et al*.[7]

We treated him with rituximab-combined CHOP (R-CHOP) : cyclophosphamide (750 mg/m^2), adriamycin (50 mg/m^2), and vincristine (1.3 mg/m^2) on day 1 and rituximab (375 mg/m^2) on day 6. After administration of rituximab, the number of WBC soon fell from 21.4×10^9/L (67% abnormal lymphocytes) to 3.7×10^9/L (20% normal lymphocytes). Therefore, we concluded that rituximab was the key drug in the treatment for this lymphoma. To date, he has received six courses of R-CHOP chemotherapy. Subsequent positron emission tomography with ^{18}F-fluorodeoxyglucose demonstrated no abnormal accumulation of ^{18}F-fluorodeoxyglucose. Gallium scintigraphy also showed no abnormal accumulation. On bone marrow examination, the abnormal lymphocytes completely disappeared and the *BCL2/IGH* rearrangement was not detected by PCR.
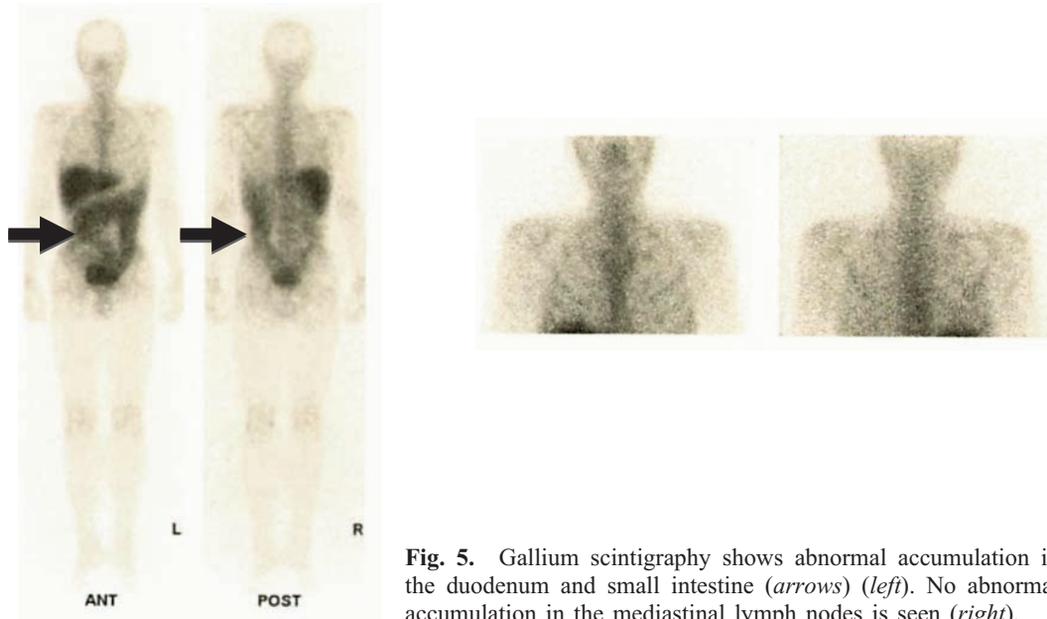
Fig. 5. Gallium scintigraphy shows abnormal accumulation in the duodenum and small intestine (*arrows*) (*left*). No abnormal accumulation in the mediastinal lymph nodes is seen (*right*).

DISCUSSION

PIFL is a provisional entity of FL, which was defined as "occurring primarily in the intestines."[1] On some occasions, it is difficult to determine the exact primary site of FL. Therefore, the criteria for the diagnosis of PIFL by Lewin[5] or Dawson[8] have been widely employed in previous reports. Lewin's criteria define PIFL as follows : lymphomas present in the intestines with abdominal symptoms that do not have other primary lesions. On the other hand, Dawson's criteria are stricter. They include (1) no palpable superficial lymph node, (2) no upper mediastinal lymph node swelling on chest X-ray, (3) no abnormal findings of white blood cells, (4) the main lesion involves the intestine and no other regional lymph nodes are enlarged under observation during surgery, and (5) no involvement of the liver or spleen. The present case satisfies almost all of these criteria except for enlarged mesenteric lymph nodes. However, it may be reasonable to consider that the mesenteric lymph node swelling was caused by direct lymphomatous invasion from the intestinal lesions. In addition, distribution of FDCs of PIFL is known to be different from that of nodal FL. In nodal FL, FDCs are densely distributed within a neoplastic follicle, while FDCs are arranged in the periphery of the follicle in PIFL.[9] The loose cluster of CD21-positive cells observed in the present patient might reflect the latter pattern of FDC distribution.

Following the report by Yoshino *et al.*,[10] duodenum-localized FL has been widely recognized by gastroenterologists and pathologists and a number of articles on it have subsequently been published. Consequently, some differences between PIFL and nodal FL have been elucidated. In a systematic review of 244 PIFL cases reported by Yamamoto *et al.*,[11] proportions of histological grades 1, 2, and 3 in PIFL were 84.4%, 11.3%, and 4.3%, while those in nodal FL were 40%, 11.3%, and 25-30%, respectively, indicating the predominance of grades 1 to 2 in PIFL. Regarding the clinical stage of PIFL in the same series, of 193 PIFL cases in which information on the clinical stage was available, only 13 and 3 cases had systemic and bone marrow diseases, respectively, indicating the predominance of early and localized stages in PIFL. Furthermore, blood involvement, including a leukemic state, was not described in a PIFL series.

Regarding PIFL tumor biology, it has been hypothesized that abnormal lymphocytes with the *BCL2/IGH* fusion gene, which circulate in the blood, adhere to intestinal mucosa and then proliferate only at this site.[12,13] In fact, Bende *et al.* demonstrated that a mucosal membrane-homing receptor protein, designated as $\alpha_4\beta_7$ integrin, is expressed on PIFL cells.[14] It is of interest that $\alpha_4\beta_7$ integrin is not expressed on nodal FL cells.

No definitive treatment for PIFL has been established.[2,11,13,15,16] Previous therapeutic modalities for PIFL have included surgical resection, radiotherapy, and chemotherapy with or without rituximab.[2,11,16] A "watchful waiting" policy has been employed in some asymptomatic patients according to their therapeutic intentions. Regarding the present patient, we treated him with R-CHOP because of leukemic disease at presentation and successfully induced complete remission.

As for recurrence of treated PIFL, 106 of 244 patients with PIFL achieved complete remission with various modalities of treatment;[11] however, in 16 of 106 patients who

achieved remission, the disease recurred 1 to 98 months later. The incidence of relapse in PIFL appears to be similar to that in nodal FL.[13] Therefore, effective therapeutic modalities for recurrent PIFL, including salvage immunochemotherapy or hematopoietic stem cell transplantation, should be established in the near future.

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