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Case Study

CD5- and CD23-Positive Splenic Diffuse Large B-Cell Lymphoma with Very Low CD20 Expression

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We report a rare primary splenic diffuse large B-cell lymphoma demonstrating $CD5^+$ and $CD23^+$ with very low CD20 expression. The only lesion was detected in the spleen, which was extremely enlarged with multiple large white-colored nodules. The lesion was characterized by a diffuse growth pattern of medium- to large-sized lymphoma cells with abundant cytoplasm. Immunohistochemical and flow cytometric study demonstrated that the lymphoma cells were negative for CD2, CD3, CD4, CD8, CD10, CD56, CD138, ALK-1, λ-light chain, and cyclin-D1, and positive for CD5, CD19, CD23, CD25, CD38, CD43, CD79a, IgM, IgD, κ-light chain, BCL2, BCL6, BOB. 1, Oct-2, Pax5, and MUM-1. CD20 was very weakly positive immunohistochemically, and negative by flow cytometric analysis. These findings resembled Richter syndrome, although chronic lymphocytic leukemia was not preexisting. Extremely poor outcome might be supposed because the effect of rituximab might be quite limited since CD20 was very weakly positive, in addition to an inferior prognosis of both $CD20^-$ and $CD5^+$ diffuse large B-cell lymphoma. Careful management is thus necessary. 〔*J Clin Exp Hematop 54(2) : 155-161, 2014*〕

Keywords: splenic lymphoma, diffuse large B-cell lymphoma, CD23, CD20, CD5

INTRODUCTION

Although malignant lymphoma occasionally involves the spleen, primary splenic lymphoma is rare. Primary splenic lymphoma is diagnosed when lymphoma infiltration is limited within the spleen and hilar lymph node (LN), without the involvement of other LNs, bone marrow (BM), or other organs.

In Japan, the most common subtype of primary splenic lymphoma is diffuse large B-cell lymphoma (DLBCL), followed by splenic marginal zone lymphoma (SMZL) and follicular lymphoma.[1]

Here, we report a rare splenic DLBCL demonstrating double positivity for CD5 and CD23, with extremely reduced expression of surface CD20.

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CASE REPORT

A 56-year-old woman was admitted to hospital because of abdominal distension and left shoulder pain. She was a carrier of hepatitis C virus without treatment. Upon admission, superficial LNs were not palpable, but an enlarged spleen was present. Only prominent splenomegaly and a swollen adjacent LN with strong ^{18}F-fluorodeoxyglucose accumulation were observed by positron emission tomography/computed tomography (Fig. 1). Laboratory findings revealed thrombocytopenia (116×10^9/L) with normal WBC (4.12×10^9/L) and RBC counts (4.36×10^{12}/L). Serum chemistry revealed elevated levels of aspartate aminotransferase (73 U/L), alanine transaminase (85 U/L), lactate dehydrogenase (555 U/L), rheumatoid factor (116 U/mL), platelet-associated IgG (123 ng/10^7 cells [normal, < 46]), and soluble interleukin-2 receptor (2,447 U/mL). C-reactive protein was negative and serum levels of IgG, IgA, and IgM were normal, without M protein or Bence-Jones protein. Splenectomy was performed, which ameliorated the symptoms and laboratory findings, and the patient was diagnosed as having splenic lymphoma. The adjacent LN could not be resected because it strongly adhered to the splenic artery. BM aspiration and biopsy demonstrated no invasion of lymphoma. We performed six courses of chemotherapy with rituximab (450 mg of rituximab on day 1, 60 mg of doxorubicin hydrochloride on day 3, 1.0 mg of vincristine sulfate on day 3, 900 mg of cyclophosphamide on day 3, and 100 mg of prednisolone on days 3 to 7).

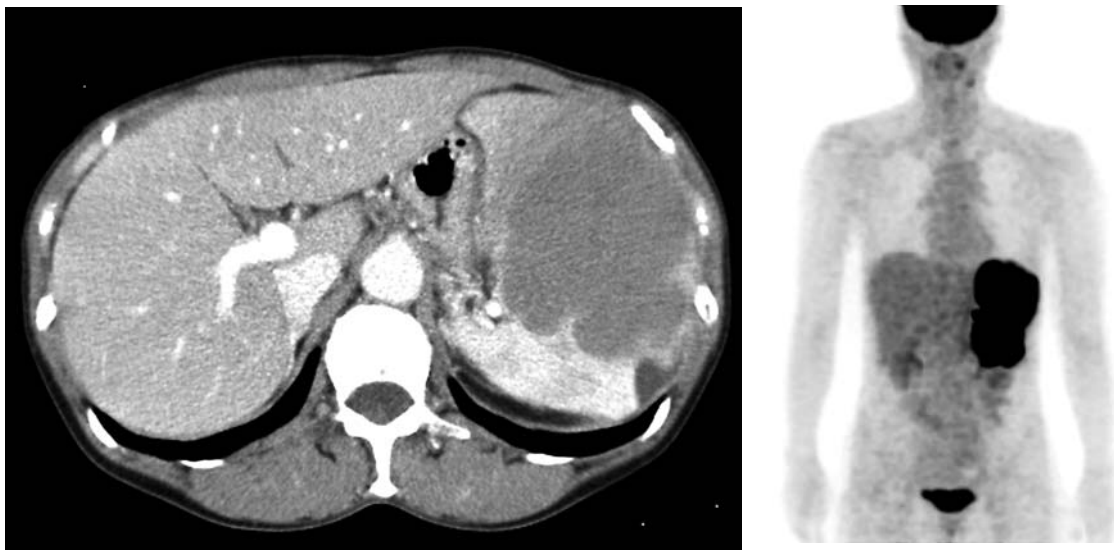

Fig. 1. Positron emission tomography/computed tomography findings. Abdominal enhanced computed tomography demonstrated a huge splenic mass (14.5 cm in diameter) of a homogeneous low-density area, which was clearly separated from normal spleen tissue. Strong ^{18}F-fluorodeoxyglucose accumulation (SUV_{max} = 21.7) was observed only in the spleen and the adjacent LN.

MATERIALS AND METHODS, AND RESULTS

Histological findings of the spleen

The spleen was extremely enlarged (930 g) and occupied by multiple large (several millimeters to several centimeters in diameter) white-colored nodules (Fig. 2a). The lesions were clearly separated from normal spleen components. At a low-power field, the lesion was characterized by a diffuse growth pattern without follicles (Fig. 2b). At a high-power field, diffuse proliferation of medium- to large-sized lymphoma cells with abundant cytoplasm was observed (Fig. 2c). A few lymphoma cells were observed within the vessels (Fig. 2d).

An immunohistochemical study (IHS) demonstrated that the lymphoma cells were negative for CD3, CD4, CD8, CD10, CD34, CD138, ALK-1, and cyclin-D1, and positive for CD5 (Fig. 2f), CD19, CD25^{low}, CD79a, IgD (Fig. 3f), BCL2 (Fig. 3c), BCL6 (Fig. 3d), and MUM-1 (Fig. 3e). Octamer-binding transcription factor 2 (Oct-2), B-cell Oct-binding protein 1 (BOB. 1), and paired box protein 5 (Pax5) were also positive (Fig. 3g-3i). CD23 and CD43 were partially positive (Fig. 3a, 3b). CD20 (Fig. 2e) was very weakly positive. The expression of IgM, λ- or κ-light chain could not be evaluated. Ki-67 was positive in 75-85% of lymphoma cells. There were no Epstein-Barr virus-encoded small RNA-positive tumor cells in the lesion by *in situ* hybridization.

Flow cytometric analysis

On flow cytometric (FCM) analysis (Fig. 4a), lymphoma cells were positive for CD5 (but were weaker than normal T cells), CD19, CD25, CD38, CD45, IgM, and κ-light chain, and negative for CD2, CD3, CD4, CD7, CD8, CD10, CD20, CD34, CD56, and λ-light chain. On the other hand, small normal B cells in the BM and peripheral blood were CD19^{+}, CD20^{bright}, and CD5^{-} (Fig. 4b).

Chromosomal analysis and fluorescence in situ *hybridization*

Chromosomal analysis was not available because of the poor condition of the cells. By fluorescence *in situ* hybridization analysis, the rate of t(11;14)(q13;q32) was 0%.

DISCUSSION

In the present case, the lesion was characterized by diffuse proliferation of medium- to large-sized lymphoma cells with abundant cytoplasm without follicles, which was compatible with primary splenic DLBCL. In IHS and FCM analysis, lymphoma cells of our patient demonstrated positive CD5 expression, which is occasionally observed in DLBCL and SMZL of the spleen.[1] In our case, the spleen was extremely enlarged and occupied by multiple large white-colored nodules. Tumor formation in the spleen is occasionally observed in DLBCL[2] and follicular lymphoma, but not in SMZL.[1] In splenic DLBCL cases with mass formation, however, IHS

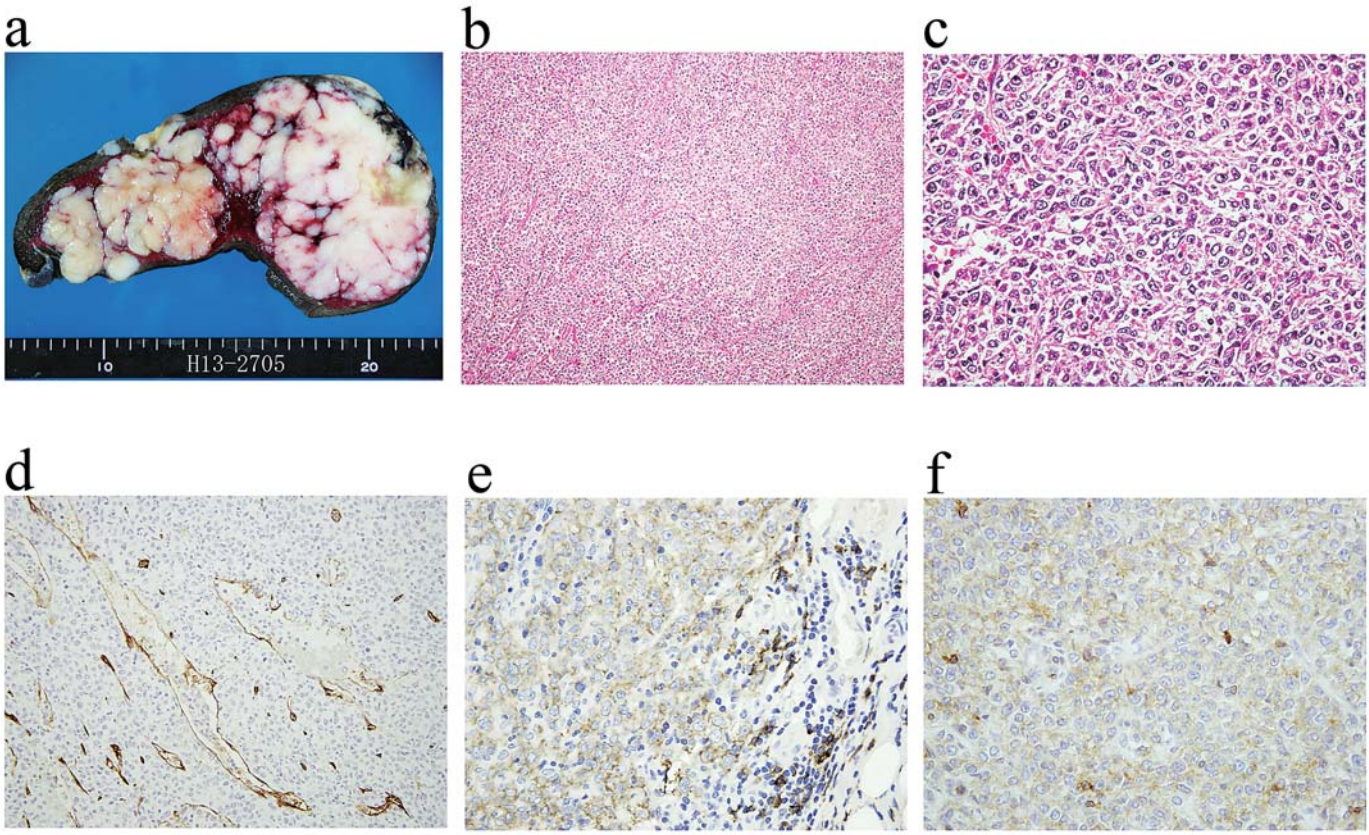

Fig. 2. Histological and immunohistochemical findings of the spleen. (***2a***) Macroscopic findings showed that the cut surface of the spleen was bulging, with multiple whitish-yellow nodular solid masses. The nodules were clearly separated from normal splenic parenchyma. (***2b***) On a low-power field, the lesion was characterized by a diffuse growth pattern of lymphoma cells without follicles. H&E × 10. (***2c***) On a high-power field, uniformly spread medium- to large-sized lymphoma cells were observed. The cells had an abundant cytoplasm with round or slightly indented nuclei and one or two prominent nucleoli. H&E × 40. (***2d***) CD34 immunostain highlighted a few intravascular lymphoma cells. × 40. (***2e***) CD20 immunostain demonstrated that the large lymphoma cells were $CD20^{dim}$. Note that the normal small B cells in the residual parenchyma were $CD20^{bright}$. × 40. (***2f***) Lymphoma cells were CD5-positive, but were weaker than normal T cells. × 40.

demonstrates $CD20^+$ and $CD5^-$.[2] Many of them show negative reactivities for CD38, CD43, BCL2, and IgD, which are distinct from the present case.

In contrast, a distinct clinicopathological entity of primary splenic lymphoma, diffuse large B-cell lymphoma manifesting in red pulp (DLBCLRP), has been reported.[3,4] Most DLBCLRP cases are $CD5^+$; however, they show positive reactivities for CD20 (bright) and IgM, and negative reactivities for CD23 and IgD. BCL6 and MUM-1 are negative in many of the cases. Moreover, many patients with DLBCLRP demonstrate infiltration to liver and BM without mass formation. From these findings, we consider that the present case is distinct from DLBCLRP.

SMZL could be ruled out by positive reactivities for CD43,[5] BCL6,[6] and MUM-1. CD43 is reported to be expressed in very early-stage B cells, lost during intermediate stages, and again expressed in plasma cells and activated B cells, being expressed in 80-95% of chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL) and mantle cell lymphoma, and 30% of DLBCL, but it is commonly absent in SMZL.[5,7] Mantle cell lymphoma was ruled out by the negative cyclin-D1 expression without t(11;14)(q13;q32) translocation.

All cases of splenic DLBCL express CD20.[1] In addition, *de novo* $CD5^+$ DLBCL has been reported to be $CD10^-$, $CD19^+$, $CD20^+$, $CD21^-$, $CD22^+$, $CD23^-$, $CD30^-$, and $CD43^+$.[8,9] On the other hand, the present case showed extremely decreased CD20 expression in IHS. Moreover, surface CD20 expression was completely negative by FCM analysis. In the case of $CD20^-$ B-cell lymphoma, plasma cell differentiation might be possible. However, the lymphoma cells were demonstrated to be positive for Pax5. Pax5 is well known to be expressed throughout the B-cell lineage, but not in cells showing plasma cell differentiation.[10-13]

It is well known that $CD5^+$ DLBCL cases show poorer prognosis than $CD5^-$ DLBCL.[8] On the other hand, patients

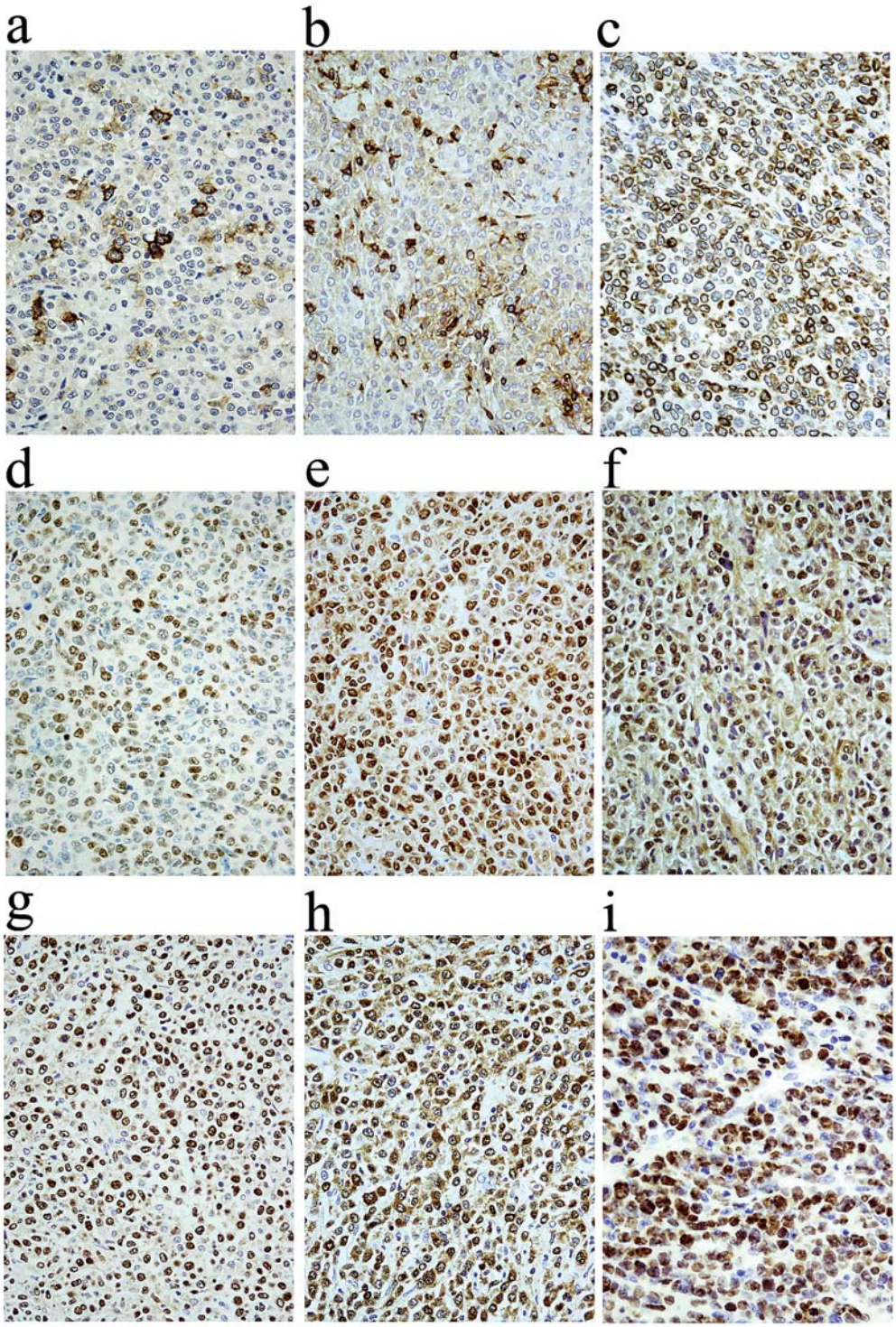

Fig. 3. Immunohistochemical findings of the spleen (continued). (***3a***) Some lymphoma cells were CD23-positive. × 40. (***3b***) A portion of lymphoma cells were CD43-positive. × 40. (***3c-3e***) The lymphoma cells had positive reactivities for BCL2 (*3c*, × 40), BCL6 (*3d*, × 40), and MUM-1 (*3e*, × 40). (***3f***) IgD was weakly positive. × 40. (***3g-3i***) The lymphoma cells were positive for Oct-2 (*3g*, × 40), BOB. 1 (*3h*, × 40), and Pax5 (*3i*, × 40).

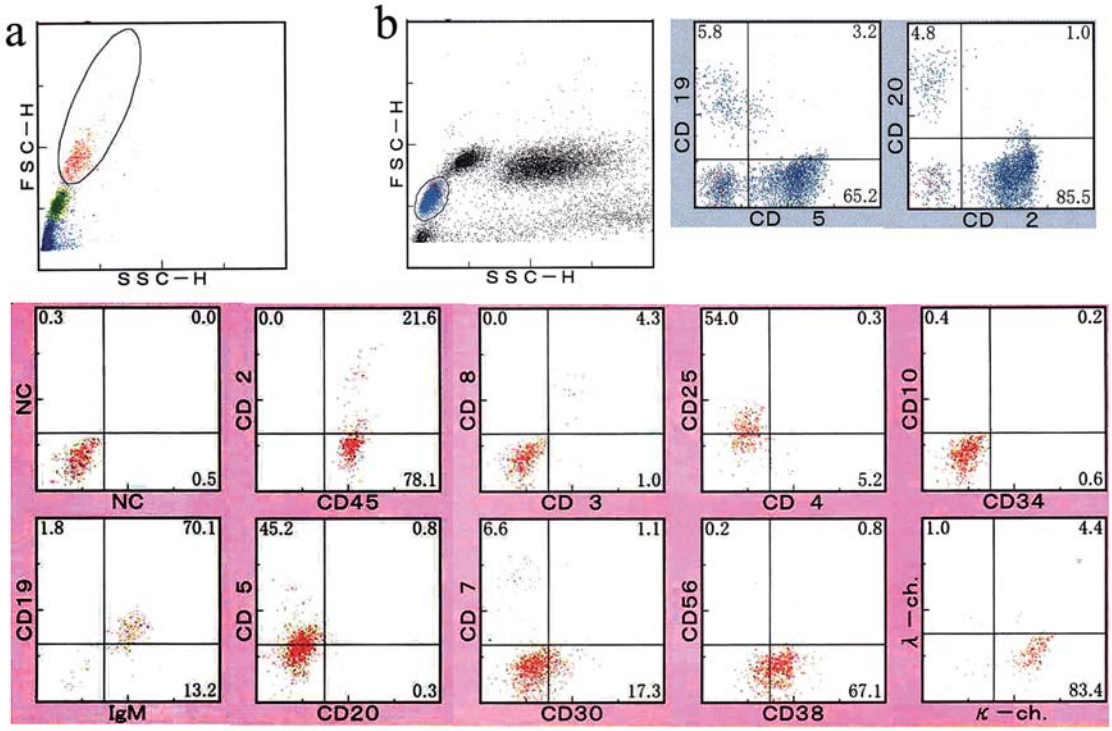

Fig. 4. Flow cytometric analysis of the spleen and peripheral blood mononuclear cells. (***4a***) Flow cytometric analysis of the spleen showed that the large cells in the spleen were CD5^{+} (weaker than normal T cells), CD19^{+}, and CD38^{+}, whereas CD10 and CD56 were negative. Surface IgM and κ-light chain were positive and λ-light chain was negative. Surface CD20 expression was completely negative. (***4b***) Flow cytometric analysis of the peripheral blood mononuclear cells showed that the normal B cells were CD19^{+}, CD20^{bright}, and CD5^{-}.

with DLBCL with negative/dim CD20 expression have markedly inferior survival.[14,15] Quite recently, it has been reported[16] that DLBCL cases showing CD20-IHS positivity and FCM negativity have significantly lower CD20 mRNA expression. The use of rituximab, however, is effective in such CD20-FCM-negative DLBCL cases. Therefore, we treated the present case with rituximab-combined chemotherapy, although the effect of rituximab might be quite limited since the patient's CD20 mRNA level was considered to be extremely low because CD20 was very weakly positive, even by IHS.

CD23 is expressed on naïve B cells in both the mantle zone and the early germinal center.[17] CD23 expression is observed in most SLL/CLL and some follicle center lymphoma cases.[18] In DLBCL as a whole, a portion (9〜16%) of cases are CD23^{+}, most of which are CD5^{-}.[18-20] In *de novo* CD5^{+} DLBCL patients, however, CD23^{+} cases are extremely rare,[4,9,21] especially in splenic DLBCL.[1,3,22]

The positive CD23 expression, together with CD5 positivity in addition to the highly reduced CD20 expression, resembled CLL/SLL[23] and Richter syndrome (RS), which is an aggressive lymphoma, mainly DLBCL, transformed from CLL.[24-27] Most cases (approximately 80%) of RS show the post-germinal center phenotype with positive reactivity for MUM-1.[28] The positive reactivities for MUM-1 and CD43 of the present case also resembled RS. No invasion of lymphoma cells into BM was confirmed by histological findings and there were no IgH rearranged bands in Southern blot analysis. In addition, lymphocytosis was not observed and the FCM study of peripheral blood mononuclear cells demonstrated the normal pattern, which ruled out the preexistence of CLL in the present case. It might be possible, however, that primary splenic SLL could transform into RS at the same time as its onset.

The prognosis of patients with RS is generally considered to be unfavorable, with the median survival being reported as 5 to 8 months.[24-27] Careful management of the present case is necessary because extremely poor outcome might be supposed as the same as RS, in addition to an inferior prognosis of both CD20^{-} and CD5^{+} DLBCL.

In conclusion, here, we report a rare case of splenic DLBCL demonstrating double positivity for CD5 and CD23, with extremely reduced expression of surface CD20. Although the preexistence of CLL was ruled out, the immunohistochemical and flow cytometric findings resembled CLL/SLL and RS. Careful management of the present case is

necessary because an extremely poor outcome might be supposed, the same as in RS.

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DISCLOSURE STATEMENT

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