

Case Report

Bone marrow infiltration of CD20-negative follicular lymphoma after rituximab therapy: a histological mimicker of hematogones and B-cell acute lymphoblastic leukemia/lymphoma

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Abstract: Rituximab is a monoclonal antibody against CD20. Rituximab combined with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy, termed R-CHOP, have improved the overall survival of patients with B-cell lymphoma in comparison with that of CHOP therapy. However, as with other molecularly-targeted therapies, resistance to rituximab could emerge sooner or later after rituximab administration. A number of mechanisms for rituximab resistance have been proposed, including downregulation of CD20 protein expression. Differential diagnosis of B-cell proliferation with reduced or lost CD20 expression includes not only B-cell lymphomas with CD20 downregulation, but also other tumorous and non-tumorous lesions. These include precursor B-cell neoplasms such as B acute lymphoblastic leukemia/lymphoblastic lymphoma (B-ALL/LBL) and hematogones, a normal precursor B-cell proliferation during regeneration of hematopoiesis, typically observed following bone marrow suppression by chemotherapy. It is important to distinguish these possibilities because distinct therapies are required for each. In this paper, we report a case where bone marrow infiltration of follicular lymphoma histopathologically mimicked hematogones or B-ALL/LBL when CD20 expression was downregulated in follicular lymphoma after R-CHOP therapy.

Keywords: Rituximab, CD20, follicular lymphoma, B acute lymphoblastic leukemia/lymphoblastic lymphoma, hematogones

Introduction

Rituximab is a monoclonal antibody against CD20. Rituximab combined with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy, termed R-CHOP, have improved the overall survival of patients with B-cell lymphoma in comparison with that of CHOP therapy [1].

However, as with other molecularly-targeted therapies, resistance to rituximab could emerge sooner or later after rituximab administration. A number of mechanisms for rituximab resistance have been proposed, including downregulation of CD20 protein expression [2]. Immunohistochemical analysis of cell lineages plays an essential part in histopathological diagnosis of lymphomas. Practically, CD20 immunohistochemistry is indispensable for detecti-

on of B-cell lineages of lymphomas. However, solitary use of CD20 immunohistochemistry may miss B-cell lymphomas with loss or downregulation of CD20 protein. To avoid this risk, it is practically important to use other B-cell markers in combination with CD20, such as CD79a.

Differential diagnosis of B-cell proliferation with reduced or lost CD20 expression includes non-tumorous lesions as well as tumorous ones. Therefore, in that context, it is important to discern whether the lesion is non-tumorous or tumorous, because distinct therapies are required for each. CD20-negative tumorous lesions contain precursor B-cell neoplasms such as B acute lymphoblastic leukemia/lymphoblastic lymphoma (B-ALL/LBL) [3] in addition to mature B-cell neoplasms such as classical Hodgkin lymphoma and around 80% of plasma cell neoplasms [4]. CD20 expression in either

CD20-negative follicular lymphoma after rituximab therapy in bone marrow

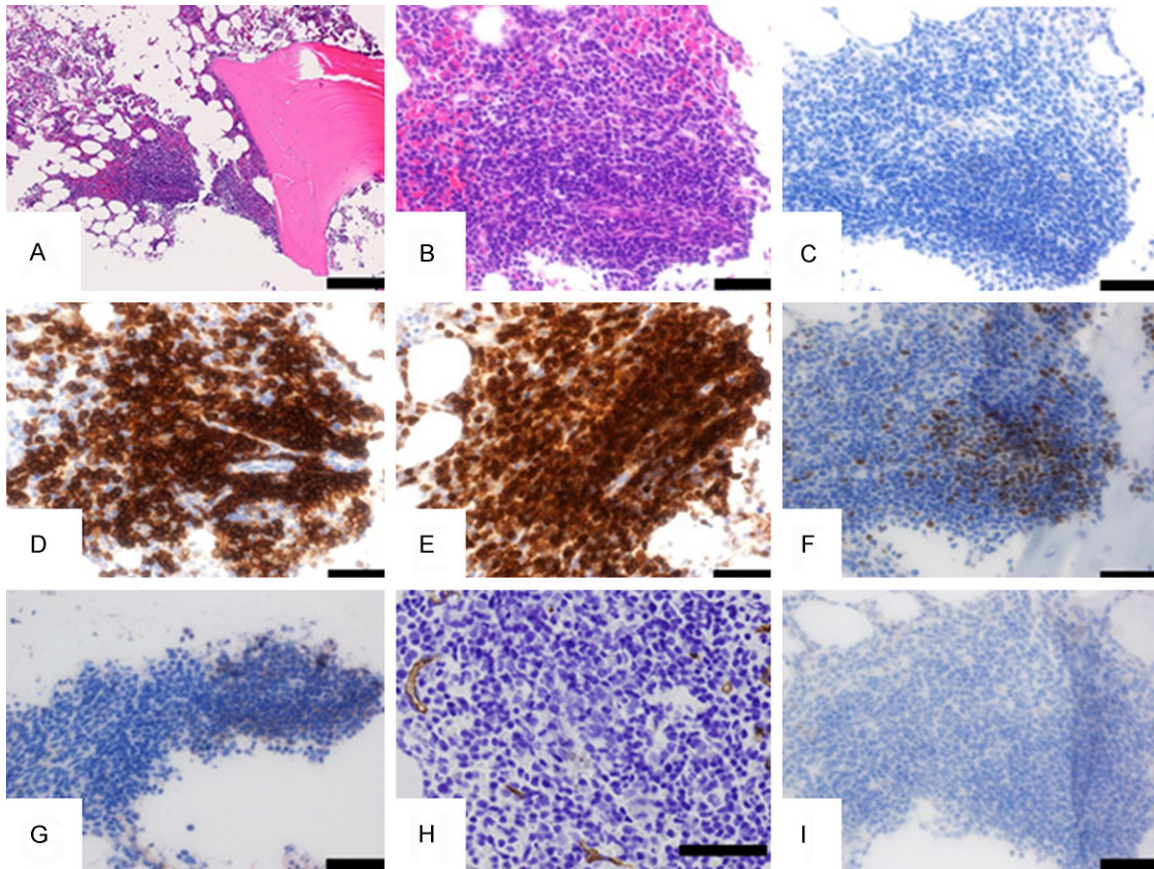


Figure 1. Representative histological images of the tumor cells in the bone marrow. (A, B) Hematoxylin and eosin (HE) stain. (C-I) Immunohistochemistry. (C) CD20. (D) CD79a. (E) BCL2. (F) BCL6. (G) CD10. (H) CD34. (I) TdT. Positive cells were stained brown in immunohistochemistry. At low power view in HE stain, the tumor cell nodules were found in the vicinity of the bone trabeculae (A). High power view showed centrocyte-like appearance of the tumor cells (B). The proliferating tumor cells were positive for CD79a (D), BCL2 (E), and BCL6 (F), weakly positive for CD10 (G), but negative for CD20 (C), CD34 (H), and TdT (I). (A) Original magnification: $\times 12.5$. Bar: 2 mm. (B-H) Original magnification: $\times 400$. Bar: 50 μm .

tumorous or non-tumorous B-cells may also be downregulated by the effects of rituximab therapy, Epstein-Barr virus (EBV) infection, or its reactivation [5, 6]. Some subtypes of classical Hodgkin lymphomas, particularly mixed cellularity subtype, are reported to be associated with EBV infection, suggesting a possible relationship between CD20 negativity of Reed-Sternberg cell and EBV infection [5, 6]. On the other hand, representatives of CD20-negative non-tumorous lesion of B-cell lineage include hematogones, a mainly precursor B-cell proliferation during regeneration of hematopoiesis, typically observed following bone marrow suppression by chemotherapy.

In this paper, we report a case where bone marrow infiltration of follicular lymphoma (FL) histopathologically mimicked hematogones or B-

ALL/LBL when CD20 expression was downregulated in FL after R-CHOP therapy.

Case report

A fifty-year-old Japanese man was referred to our hospital for the examination of progressive swelling of systemic lymph nodes. Fourteen years before, the patient had been referred to another hospital for examination of multiple swollen lymph nodes of 1 to 2 cm in bilateral cervical, right axillary, and bilateral inguinal regions. At that time, computerized tomography (CT) imaging of the abdomen showed swelling of hepatic portal lymph nodes and splenomegaly as well. Histopathological examination of the lymph node and bone marrow biopsy led to a diagnosis of peripheral T-cell lymphoma and its involvement in the bone marrow (data not

shown). Based on this diagnosis, combination chemotherapy using etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone, and bleomycin, was administered but ineffective. Reexamination of the histology and the flow cytometry data of the original lymph node biopsy resulted in the correction of the diagnosis as FL and its involvement in the bone marrow (data not shown). He was administered rituximab for subsequent 6 years, which led to complete remission (CR) that was maintained for the following 2 years. However, the disease relapsed with progressive swelling of systemic lymph nodes and appearance of lymphoma cells in the peripheral blood. He was administered 4 courses of R-CHOP and 2 courses of R-COP, resulting in the second CR, which was followed up with rituximab maintenance. However, the disease relapsed again 5 months before. The positron emission tomography/CT showed progressive enlargement of lymph nodes of around 10 mm in diameter in bilateral axillary, mediastinal, para-aortic, and inguinal regions. In addition, it revealed progressive splenomegaly of around 150 mm in size and soft tissue density in the bone marrow, presumably caused by bone marrow involvement. This time the patient was treated with bendamustin combined with rituximab, followed by rituximab maintenance, which was considered to be partially effective: on admission to our hospital at this time, his serum biochemistry showed decrease in lactate dehydrogenase to 228 U/L (normal range: 119-229) and decrease in soluble interleukin-2 receptor from 8000 to 1239 U/mL. Bone marrow biopsy was performed to examine the possibility of lymphoma cell infiltration.

HE staining of the bone marrow biopsy specimen showed several foci of nodular proliferation of monotonous small lymphoid cells adjacent to the bone trabeculae (**Figure 1A** and **1B**). This paratrabeular distribution of small lymphoid nodules was considered to be consistent with relapse and bone marrow infiltration of the past FL. However, immunohistochemical examination revealed that the proliferating lymphoid cells were stained unexpectedly negative for CD20 (**Figure 1C**), although they were positive for CD79a (**Figure 1D**). As small B lymphoid proliferation with CD20 loss, the differential diagnosis included FL with CD20 downregulation after rituximab therapy, B-ALL/LBL, and hematogones. Subsequent immunohistochemical analysis showed that the proliferating B lymphoid

cells were positive for BCL2 (**Figure 1E**) and BCL6 (**Figure 1F**), weakly positive for CD10 (**Figure 1G**), but negative for CD34 (**Figure 1H**) and terminal deoxynucleotidyl transferase (TdT) (**Figure 1I**). The diagnosis of infiltration of CD20-negative FL in the bone marrow was thus confirmed and established.

After splenectomy, the patient underwent haploidentical hematopoietic stem cell transplantation 10 months after the above diagnosis. The follow-up CT after the transplantation showed no significant swelling of lymph nodes in thoracic and abdominal cavities.

Discussion

In this paper, we report a case of bone marrow infiltration of CD20-negative FL after rituximab administration, which histologically mimicked B-ALL/LBL or hematogones. There are some case reports on CD20 downregulation or loss in B-cell lymphomas after rituximab therapy [7-10]. A case of CD20-negative diffuse large B-cell lymphoma was reported that was transformed from FL after rituximab therapy [7]. Presumably, rituximab-resistance due to CD20 loss may increase aggressiveness of the lymphoma, together with transformation into diffuse large B-cell lymphoma. Thus, the progressive swelling of the lymph nodes after the chemotherapy in the present case made us suspicious of emergence of the chemo-resistance or transformation into aggressive lymphoma of the original FL. However, to our knowledge, there were no reports or discussion on the potential diagnostic challenge that loss of CD20 poses in the context of bone marrow infiltration of B-cell lymphoma.

In the present case, the past clinical history of FL readily allowed us to consider bone marrow infiltration of FL in the first place. In HE stain, the paratrabeular infiltration by lymphoid cells was suggestive of lymphoma infiltration into the bone marrow. However, immunohistochemical examination revealed that the proliferating lymphoid cells were stained unexpectedly negative for CD20, although they were positive for CD79a. Thus, we needed careful consideration for the correct diagnosis.

In general terms, the differential diagnosis of proliferation of CD20-negative and CD10-positive lymphoid cells includes not only CD20-negative and CD10-positive FL but also B-ALL/

LBL as a tumorous lesion and hematogones as a non-tumorous lesion. B-ALL/LBL is a neoplasm of CD20-negative precursor B lymphoblastic cells expressing TdT and CD34 in addition to CD10. Hematogones are non-tumorous proliferation of mainly precursor B-cells in the bone marrow [11-13]. The amount of hematogones increases in regenerative phase of the bone marrow, including various cytopenic situations or neoplastic processes [11]. Flow cytometry analysis indicated that hematogones are heterogeneous mixture of B-cells mostly at immature stage, composed of a small population of very immature B-cells with TdT and CD34 expression, a predominant fraction of CD10-positive and TdT-negative B-cells, and substantial fraction of CD20-positive mature B-cells [14]. It was reported that BCL2 expression was useful to distinguish FL from hematogones [15]. In the present case, immunohistochemical analysis revealed that the proliferating B lymphoid cells were positive for BCL2 and BCL6 but negative for CD34 and TdT. CD10 was partially and weakly positive. These results were consistent with the diagnosis of CD20-negative FL.

For other possibilities, coexistence of splenomegaly and bone marrow involvement suggested hairy cell leukemia as a rare but possible differential diagnosis, which was ruled out by negative immunohistochemistry for cyclin D1 (data not shown), and by the presence of massive lymph node involvement [16]. Last but not the least, it could not be excluded that the lymphoma observed in the bone marrow biopsy is a 'primary' lymphoma of the bone. Immunohistochemically, less than 50% of primary non-Hodgkin lymphoma of the bone is CD10-positive, more than 50% being BCL6-positive and/or BCL2-positive [17]. Thus, without appropriate clinical information, it may be difficult to distinguish bone marrow infiltration of FL from primary non-Hodgkin lymphoma of the bone. Taken together, the diagnosis of infiltration of CD20-negative FL was thus established.

What are the mechanisms for CD20 downregulation or loss in the B-cell lymphomas after rituximab therapy? A number of mechanisms for rituximab resistance have been proposed, including downregulation of CD20 protein expression [2]. In an extreme case, deletion of CD20 gene led to loss of CD20 expression [18]. In another extreme case, CD20 was reported to

be re-expressed in a reversible fashion in B-cell lymphomas that had once lost CD20 expression [2, 19]. Presumably, epigenetic mechanisms such as DNA methylation-based CD20 promoter silencing may be involved in this case of reversible CD20 expression [2]. In the near future, rituximab resistance due to CD20 downregulation may be partly overcome by epigenetic therapies using inhibitors of DNA methylation and so on.

In conclusion, as illustrated in the present case, bone marrow infiltration of FL may mimic B-ALL/LBL and hematogones when CD20 expression in FL is downregulated or lost. The background for CD20 downregulation may include chemotherapy containing rituximab. In order to avoid this pitfall and reach to a correct histopathological diagnosis of the bone marrow specimen, clinical information of the patients is critical on previous history of FL or its therapy, if any. Without such information, bone marrow infiltration of CD20-negative FL would be a diagnostic challenge to histopathologists. Previously, we reported a case of FL mimicking nodal marginal zone lymphoma as a potential pitfall for FL diagnosis [20]. The CD20-negative FL after rituximab therapy in this paper is another diagnostic pitfall for FL.

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Disclosure of conflict of interest

None.

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