

Disease progression after R-CHOP treatment associated with the loss of CD20 antigen expression

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Conflict-of-interest disclosure:
 The authors declare no competing financial interest

Submitted: 4/8/2010
 Accepted: 12/22/2010

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DOI: 10.5581/1516-8484.20110036

A case of a follicular lymphoma transformed into a CD20⁺ is described which progressed with the loss of CD20 expression after 8 cycles of R-CHOP. This phenomenon is not a rare event and has shown poor prognosis. Our purposes are to describe this event and suggest biopsy in relapsed or progressive disease

Keywords: Lymphoma, Large B-cell, diffuse; Disease progression; Immunotherapy

Introduction

The use of rituximab – a chimeric monoclonal antibody against CD20 protein - has become a molecular target treatment for CD20⁺ B cell non-Hodgkin lymphoma (NHL). Rituximab associated with chemotherapy has been indicated for low and high grade B cell NHL and for the maintenance of relapsed follicular lymphoma. The R-CHOP protocol (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone) is currently the gold standard treatment for diffuse large B cell lymphoma (DLBCL). Although this protocol attains the best rates of complete response (76%)⁽¹⁾ and 4-year overall survival (55 to 94%),⁽²⁾ there is a considerable number of patients that are refractory to treatment or present early relapse. The relapse and overall survival rates depend on risk factors. Progression-free survival at 4 years for patients without risk factors, with 1 or 2 risk factors and with 3 or more risk factors is 6%, 20% and 47%, respectively.⁽²⁾

Studies demonstrate that individual characteristics can influence the response of rituximab against CD20⁺ cells, such as the Fc gammaRIIIa-158V/F polymorphism,⁽³⁾ expression of the CD20 antigen⁽⁴⁾ and CD20-positive tumor burden.⁽⁵⁾

Currently, it is extremely difficult to define resistance to rituximab, however some mechanisms have been described with unclear clinical meanings such as: the loss of CD20 antigen expression, mechanisms of antigen-antibody binding interactions and the expression of inhibitors including CD55 and CD59 antigens, among others.⁽⁶⁻⁸⁾

Recently, loss of CD20 expression was demonstrated in refractory and/or relapsed cases treated with rituximab-based chemotherapy. This fact should be considered carefully, since almost all second line protocols are based on the association of rituximab with chemotherapy (R-ICE and R-DHAP). Our purpose is to describe the case of a follicular lymphoma transformed into CD20⁺, which progressed with the loss of CD20 antigen expression after an R-CHOP regimen.

Case Report

In September of 2008, a 58-year-old woman presented with a follicular lymphoma transformed into CD20⁺, stage IV XEB (bone marrow infiltration, retroperitoneal bulky disease, pleural infiltration), with three risk factors (clinical stage IV, elevated lactate dehydrogenase and more than one extra nodal site). The biopsy of an abdominal lymph node identified DLBCL while the bone marrow biopsy was characterized by follicular lymphoma infiltration.

She was treated with pre-phase therapy (cyclophosphamide 600 mg/m² day 1, vincristine 1.4 mg/m² day 1 and prednisone 100 mg days 1-5) followed by 8 cycles of R-CHOP given every 21 days. Ten days after the last cycle of chemotherapy, subcutaneous nodules appeared in the abdomen with infiltration in the skin. Fine needle aspiration biopsy and skin biopsy were performed that identified infiltration of the skin by diffuse atypical monomorphic medium to large-sized lymphocytes. Immunohistochemistry demonstrated a

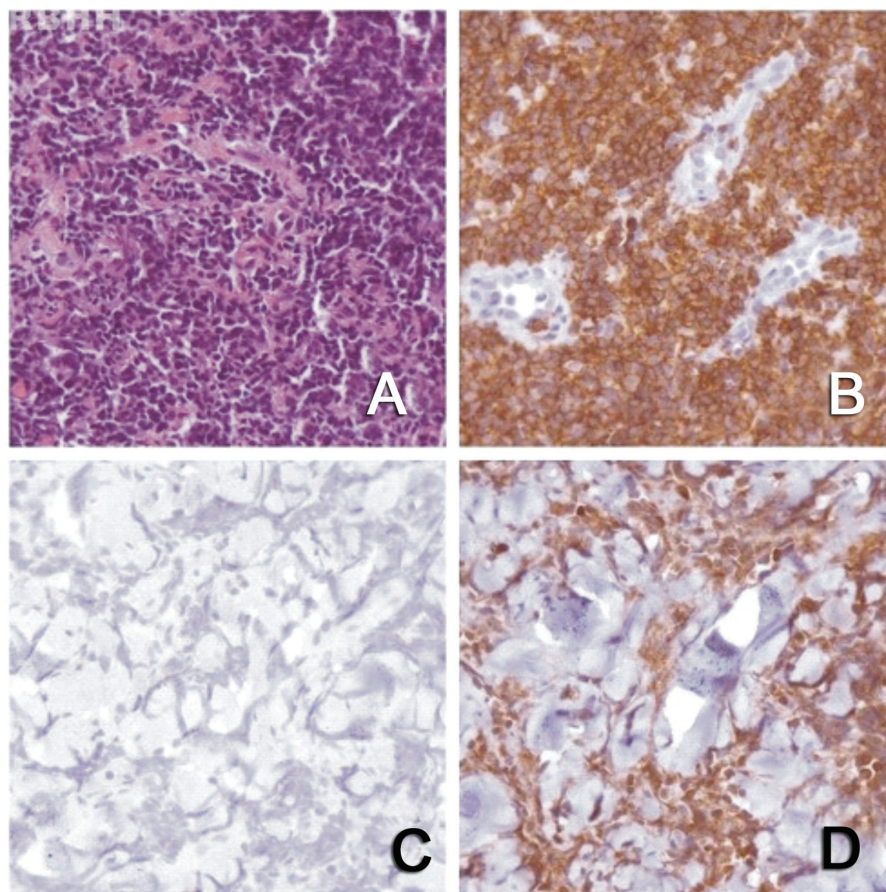


Figure 1 A and B – CD20⁺ Diffuse large B cell lymphoma at diagnosis
Figure 1 C and D – CD20⁻ diffuse large B cell lymphoma on relapse (C) and CD79a positive (D)

CD20-negative antigen, a CD79a-positive antigen, a high positive Ki-67 index and a focal CD3-positive antigen that was compatible with progressive disease characterized by the CD20-positive antigen expression; this phenomenon was confirmed by flow cytometry.

The patient started treatment in April 2009 with the IVAC protocol (ifosfamide, etoposide and cytarabine)⁽⁹⁾ and 2 cycles of high-dose methotrexate (3.5 g/m²), but she was refractory. She died in September 2009 five months after confirmation of progression.

Discussion

This case shows the loss of CD20-antigen expression during the evolution of a transformed lymphoma. This phenomenon raises some questions: what is the real incidence of this event? How do these new CD20-negative lymphomas behave? Are we searching for the diagnosis? What are the biological findings of this new entity? Currently, these questions cannot be answered.

There are few reports in the literature about the loss of CD20 antigen expression after treatment with rituximab. These

studies are not homogeneous and do not have a prospective methodology. There are two main studies that try to estimate the incidence of this phenomenon, however according to the authors, because they did not carry out biopsies on all relapsed patients and those with progressive disease, the analysis of the frequency of CD20-antigen loss is extremely inaccurate.^(10,11) Kennedy et al. carried out biopsies on ten of 13 relapsed cases; CD20-antigen loss was found in six (46%).⁽¹⁰⁾ Hiraga et al. performed biopsies in nineteen of thirty-six relapsed cases; five cases had lost the CD20-antigen expression corresponding to 14% of all relapses and two had transformed follicular lymphoma.⁽¹¹⁾ In most cases, performing a further biopsy in relapsed or progressive disease cases was related to an aggressive or unusual presentation⁽⁹⁾ as in our report, where the relapse was characterized by subcutaneous nodules and skin infiltration that culminated in the patient's death just five months after relapse and one year after diagnosis. Higara et al. also had 100% of mortality in five cases with maximum survival of eleven months after the confirmation of CD20-antigen loss.⁽¹¹⁾

There are some explanations for the pathogenesis of CD20-antigen loss in CD79a-positive lymphomatoid B cells.

First, it may be explained by a virtual blockade of all CD20-antigen sites binding to rituximab.^(7,8) Second, the existence of CD20-negative cells could have been positively selected by rituximab. On the other hand, CD20-positive B cells might have mutated leading to loss of expression⁽¹²⁾ resulting in the absence of transcription or the internalization of the CD20 antigen.

A possible explanation of CD20-antigen loss was demonstrated by Higara et al. using B cell lineages derived from CD20-negative relapsed patients. The authors showed that the *in vitro* treatment of these cells using 5-aza-2'-deoxycitidin stimulated gene expression resulting in higher levels of CD20 Mrna.⁽¹¹⁾

This case illustrates the importance of carrying out further biopsies during the evaluation of relapsed or progressive B cell NHL who are candidates for rituximab rescue therapy. Moreover, the loss of CD20-antigen expression seems to be related to a poor prognosis. Thus, more studies are necessary to elucidate the real incidence and importance of this phenomenon in the follow-up of B cell NHL treated with rituximab.

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