Re-occurrence of the CD20 molecule expression subsequent to CD20-negative relapse in diffuse large B-cell lymphoma

We report the first case of diffuse large B-cell lymphoma (DLBCL) of the stomach displaying CD20-negative relapse after rituximab-containing treatment and the re-appearance of CD20 expression at the second failure. The loss of CD20 expression in B-cell lymphomas relapsing after rituximab is a well-known phenomenon, but its actual impact in DLBCL is difficult to estimate. This paradigmatic case suggests that CD20-expression reappearance after purging of CD20-positive clones with rituximab might be an underestimated occurrence in B-cell lymphomas. Accordingly, every relapse, whenever possible, should be histologically assessed with diagnostic and immunophenotyping purposes.

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Case report

A 85-year-old male with a history of hepatitis C virus infection was admitted at Our Institution with a diagnosis of diffuse large B-cell lymphoma (DLBCL) of the stomach (Ann Arbor stage IEA). Immunophenotypic analysis on diagnostic samples obtained by multiple gastroscopic biopsies showed CD20 expression (L26 clone) in 100% of neoplastic cells (Figure 1a). The patient was treated with a conservative strategy consisting of four courses of chemo-immunotherapy with rituximab 375 mg/m^2 on day 1, cyclophosphamide 600 mg/m^2 on day 1, epidoxorubicin 50 mg/m^2 on day 1, vincristine 2 mg on day 1, prednisone 50 mg daily on days 1 to 5, every 28 days. A pathologically documented complete response was obtained since the second course. At six months from therapy conclusion, a local relapse, consisting of a 5-cm ulcer in the posterior wall of the stomach, was found. The evaluation of nine gastric biopsies showed a diffuse proliferation of CD20 (L26)-negative/CD79a-positive (Figure 1b) large cells. CD20-negative phenotype was confirmed with an additional anti-CD20 monoclonal antibody reactive against the C-terminal intracellular domain of the transmembrane CD20 molecule (7D1 clone, Novocastra, Newcastle, UK). The second-line therapy was 39.6 Gy irradiation to the stomach and regional lymph nodes, thus obtaining a second pathological complete remission. At six months from the last relapse, a further local recurrence consisting of a large gastric ulcer was diagnosed. Histological evaluation of multiple biopsies showed a picture of DLBCL with the expression of CD20 molecule in 10% of neoplastic cells; CD20 immunoreactivity was detected by using both L26 and 7D1 monoclonal antibodies (Figure 1c). It was not possible to start a new treatment since the patient's general conditions gradually worsened; he died of progressive lymphoma at 16 months from original diagnosis.

Discussion

This case displays two unreported features. To the best of our knowledge, this is the first case of gastric DLBCL displaying CD20-negative relapse after rituximab treatment. The loss of CD20 expression in non-Hodgkin lymphomas (NHL) relapsing after rituximab is a well-known phenomenon. This occurrence has been interpreted as a mechanism of drug resistance to this drug and, likely, due to the selection of pre-existing CD20-negative neoplastic cells or to the induction of mutated CD20-negative clones[1]. The actual impact of

Figure 1: A) Diffuse large B-cell lymphoma of the stomach; CD20 immunoreactivity evidenced in 100% of neoplastic cells by L26 clone monoclonal antibody. B) Histopathological picture at relapse, resulting in CD20 (L26)-negative phenotype; CD79a immunoreactivity (insert) confirms the B-cell immunophenotype of neoplastic cells. C) CD20 immunoreactivity (arrows) in near 10% of neoplastic cells at the second relapse, detected by using L26 clone monoclonal antibody. The use of a second antibody against the C-terminal intracellular domain of the transmembrane CD20 molecule (7D1 clone, Novocastra, Newcastle, UK) displayed overlapping results when compared with L26 antibody (data not shown). All immunohistochemistry techniques were performed using Novolink Polymer Detection Kit (Novocastra, Newcastle, UK) according to manufacturers' instructions.

this phenomenon is difficult to estimate[2-4] since the reported prevalence oscillates between 0%[3] and 46%[5] of relapses; they are mostly described in follicular lymphomas,[1,5] rarely in DLBCL[6]. These discordant prevalence figures may be due to the different assays employed for the detection of CD20 molecule. Rituximab itself, by virtue of its blocking CD20-binding sites, which lasts up to 6 months, can result in an apparent CD20 negativity at flow cytometry[3]. Accordingly, the immunostain with L26 antibody, which recognizes the CD20 intracellular domain, should be performed to define CD20 negativity[7], while CD79a, another B-cell marker, may be a useful tool in the identification of lymphomatous CD20-negative (L26-negative) B-cells in some particular settings.[2,8] Our patient experienced a real CD20-negative relapse, considering that tumor cells displayed an L26-negative/7D1-negative/CD79a-positive pattern. These histopathological features are reliable, since they were carried out on several biopsies, and the immunophenotype of tumor cells and internal positive controls (small B-lymphocytes; Figure 1b) was constant among different bioptic samples.

The second and most intriguing aspect of our case is the re-appearance of CD20 expression at the second failure, following a previous CD20-negative relapse. No other cases with similar characteristics have been reported in literature; this depends on the fact that patients with multiple relapses of nodal or extranodal NHL are rarely referred to systematic surgical biopsy, while this latter strategy is a current practice during the follow-up of gastric lymphomas. This paradigmatic case suggests therefore that CD20-expression reappearance after purging of CD20-positive clones with rituximab might be an underestimated occurrence in B-cell lymphomas. Accordingly, every relapse, whenever possible, should be histologically assessed with diagnostic and immunophenotyping purposes. In particular, re-immunophenotyping may have relevant therapeutic implications considering that the loss of CD20 expression prevents to retreat these patients with rituximab or radiolabeled anti-CD20 antibodies as salvage therapy. Conversely, the CD20-expression reappearance, and a progressively further increase in CD20-positive cells amount, could allow the use of anti-CD20 therapy (mostly radiolabeled monoclonal antibody) once again. This is a very important issue since re-treatment with rituximab has been associated with a 42% remission rate and a median progression-free survival of 20 months;[9] while the use of ^{90}Y-ibritumomab tiuxetan, an anti-CD20 radioimmunoconjugate, resulted in a 74% response rate in rituximab-refractory lymphomas.[10] In conclusion, our findings support the strategy to perform biopsy with CD20 immunostaining in any patient with relapsed lymphoma, potentially eligible for anti-CD20 therapy. Since its relevant therapeutic implications, this diagnostic strategy should be strongly recommended in NHL patients.

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