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CD20 positivity in classical Hodgkin's lymphoma: Diagnostic challenge or targeting opportunity

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Abstract

Background: It is now well established that Hodgkin cells are clonal B cells with a CD30 and CD15 phenotype. However, on immunohistochemistry, in our experience and the experience of others, CD20 positivity in an otherwise typical classical Hodgkin's Lymphoma is not uncommon and if associated with CD15 negativity poses a potential diagnostic trap and is likely to be called B-NHL. **Objective:** To assess the frequency of B-cell related antigens CD20 and CD79a in classical Hodgkin's Lymphoma. **Materials and Methods:** A total of 91 consecutive cases of classical Hodgkin's Lymphoma were analyzed for co-expression of CD20 and CD79a. Both males and females of all ages were included in this study. All cases of nodular lymphocyte predominant Hodgkin's Lymphoma were excluded. All the cases were stained with a panel of antibodies including LCA, CD20, CD79a, CD30, CD15, CD3, EMA and Alk. Protein. **Results:** All 91 cases of classical Hodgkin's Lymphoma showed negativity for LCA and positivity for CD30. Eighteen cases (19.8%) showed distinct membrane staining with CD20 in most of the large atypical cells. However, out of these, only 7 cases (7.7%) showed CD79a co-expression, which was largely focal. CD15 negativity with CD20 positivity was seen in 7 (7.7%) cases of otherwise typical classical Hodgkin's Lymphoma. **Conclusions/Recommendations:** CD20 expression is frequent in classical Hodgkin's Lymphoma and our results are in consensus with reported literature on this subject. In these cases, LCA negativity of large cells was extremely useful in clinching the right diagnosis.

Keywords: Hodgkin′s lymphoma, immunohistochemistry

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Introduction

Hodgkin's Lymphoma (HL), formerly known as Hodgkin's disease, is a type of lymphoma first described by Thomas

Hodgkin in 1832. [1] HL is characterized clinically by the orderly spread of disease from one lymph node group to another and by the development of B symptoms with advanced disease. Pathologically, the disease is characterized by the presence of Classic Reed-Sternberg cells More Details and its variants [Figure 1]. HL was one of the first cancers to be rendered curable by combination chemotherapy, hence diagnostic accuracy. In almost all classical HL cases, neoplastic cells express CD30 and approximately 70% of these co-express CD15. [2]

The vast majority of HL, both classical Hodgkin's lymphoma (cHL) and nodular lymphocyte predominant Hodgkin's lymphoma (NLPHL), are known to be of B-cell origin.[3] However, the reasons for incomplete development of the B-cell phenotype and lack of immunoglobulin expression in cHL have not been explained fully. Crippling immunoglobulin gene mutations are suggested to be the cause of absent immunoglobin expression in cHL. [4] However, recent work has focused on the impaired activation of the immunoglobulin promoter secondary to defective interaction with or lack of expression of transcription factors as the dominant mechanisms. [5]

However, in our experience and the experience of others, a number of cases of cHL do show positivity for B-cell markers and in particular for CD20. This adds to diagnostic difficulties, particularly with these cases because they include T-cell rich B-cell lymphoma (TCRBCL), anaplastic large-cell lymphoma (ALCL) and peripheral T-cell lymphoma (PTCL). However, on the other hand, this expression of CD20 may be exploited for anti-CD20 therapy.

Aim of Study

The aim of this study is to assess expression of the B-cell-related antigens CD20 and CD79a in cHL by implying a comprehensive antibody panel.

Inclusion criteria

Only the cases of cHL were included in the study.

Exclusion criteria

The cases of NLPHL were excluded from the study.

Materials and Methods

A total of 91 consecutive biopsy-proven cHL cases were included in the study. All cases were stained with a panel of antibodies including the following:

LCA (CD45 clone 2B11 + PD7/26, monoclonal mouse anti-human Dako cytomation)

CD20 (clone L26 monoclonal mouse anti-human CD20cy Dako cytomation)

CD79a (clone JCB117 monoclonal mouse anti-human CD79a Dako cytomation)

CD3 (polyclonal rabbit anti-human CD3 Dako cytomation)

CD30 (clone Ber-H2 monoclonal mouse anti-human CD30 Dako cytomation)

CD15 (CD15 clone SPM490 mouse monoclonal antibody, Thermo Scientific, Lab Vision Corporation U.S.A.)

EMA (clone E29 monoclonal mouse anti-human epithelial membrane antigen)

ALK Protein (clone ALK 1 monoclonal mouse anti-human CD246 ALK protein Dako cytomation)

All tissue samples were fixed in 10% buffered formalin processed routinely and embedded in paraffin. Sections of 3-4 µm were cut and mounted on positively charged super frost slides and dried in an oven for 1 hour at 60ºC. The sections were then deparaffinized in xylene and rehydrated in graded ethanol. For epitope retrieval, the tissue sections were heated in Tris EDTA (pH 9.0) for 16 minutes at 450 W in a scientific microwave oven. After the heat-induced epitope retrieval procedure, the sections were cooled down gradually to room temperature and washed with running tap water. Endogenous peroxidase was blocked by incubating the tissue in 3% hydrogen peroxide at room temperature for 10 minutes and washed with distilled water. The tissue samples were incubated with primary antibodies for 30 minutes at room temperature. After incubation with primary antibodies, immune detection was performed with the 2-step polylabeling method using the envision system (Dako) with diaminobenzidine (DAB) chromogen as a substrate. After DAB, the slides were counter stained by hematoxlin and finally were mounted with distrine-pthaline xyline (DPX).

Results

Out of 91 cases of cHL, 70 cases were from males and 21 were from females. Of these 91 cases, 40 (44%) cases were of

the cHL nodular sclerosis type, 47 (51%) were of the cHL mixed cellularity type and 4 (4.4%) cases were of the cHL lymphocytic rich type. All these 91 cases (100%) showed negativity for LCA and positivity for CD30 [Bar Chart 1]. The staining for CD30 was predominantly membranous with perinuclear golgi positivity in most cases [Figure 2]. Out of 91 cases, 84 cases (92.3%) also showed membranous and or golgi positivity for CD15 [Figure 3] and 18 cases (19.8%) showed distinct membrane staining with CD20 [Figure 4] in most of the large atypical Hodgkin's cells. In relation to the subtypes of classical Hodgkin's lymphoma, 8 out of 40 (20%) were nodular sclerosis, 8 out of 47 (17%) were mixed cellularity cases and 2 out of 4 (50%) cases of lymphocytic rich type showed positivity for CD20 [Table 1]. Of the 18 cases showing CD20 positivity, only 7 cases (7.7%) also showed CD79a co-expression, which was largely focal and membranous [Figure 5]. None of the CD20 negative cases showed CD79a positivity. CD15 negativity with CD20 positivity was seen in 7 (7.7%) cases of otherwise typical cHL. The background reactive population was mostly composed of mature T cells intermixed with histiocytes, plasma cells and polymorphs (neutrophils and eosinophils).

Discussion

In practice, the diagnosis of Hodgkin's Lymphoma rather seems straightforward but cells resembling Reed-Sternberg cells have been described in various other entities including Non-Hodgkin's Lymphoma (NHL) and virus associated lymphadenopathies. Immunohistochemical stains, in particular CD30 and CD15, are used routinely for confirmation and resolve difficult cases. [2]

Problems arise, however, in those cases where CD15 is either negative or only focally positive with strong diffuse membranous CD20 positivity. In these cases, large B cell lymphoma (T cell /histiocytic rich) also comes into differential diagnosis as this tumor usually also co-expresses CD30 (an activation marker not specific for HL) and rarely CD15 as well. In this situation, we go back to time tested H and E to seek further clinical information in particular about the pattern of disease. In addition, in these cases we also critically evaluate the LCA pattern as positivity in classical HL is highly unusual. Antibodies for EBV like LMP are also useful as many cases of classical HL do show nuclear staining with this antibody.

In this study, we have seen about 20% reactivity with CD20 in cHL in contrast with the German lymphoma study group. von Wasielewski *et al* . [6] has reported a frequency of CD20 positivity of 4.8% among 1751 patients with cHD. In another study by Portlock *et al* . in 2004, 28 (11%) out of 248 cases showed CD20 positivity with the distinct membrane staining; however, occasional golgi zone staining was also observed, which was not seen in our study. [7]

In this study, only 7 cases (7.7%) showed CD79a co-expression, which was largely focal and membranous. Our findings were in agreement with the finding observed by Tzankov *et al* . [8] Tzankov observed that out of 330 cases CD20 was expressed in 25% of cases and CD79a was observed in 7% of cases, respectively. CD20 and 79a were co-expressed in 16 cases (P <0.005).

George *et al* . [9] observed that CD20 was expressed by Hodgkin and Reed-Sternberg cells in 22% of patients. In another study, Tzankov *et al* . [10] observed that Hodgkin's and Reed-Sternberg cells expressed CD20 antigen positivity in 20% (24 of 119) of the cases. In these studies, the percent of CD20 positivity was in agreement with our study.

More recently, interest in using anti-CD20 in cHL expressing CD20 has been revived; however, its utility in bigger trials is yet to be seen. Monoclonal antibody IDEC-C2B8 (Rituximab) has been shown to be highly effective in the treatment of NHL. [11] More intensive doxorubicin containing regimens have been given along with Rituximab (anti-CD20 antibody) to patients showing CD20 positivity in RS cells. [12]

In another study, a 375 mg/m ^{2} dose of Rituximab was given once a week for 4 weeks to a patient who had relapsed after six cycles of doxorubicin-bleomycin-vinblastin-dacarbazine (ABVD) and cyclophosphamid-vincristine-procarbazine-prednison (COPP) regimens. Treatment was well tolerated and a complete remission was achieved 2 months later. [13]

Kirchner *et al* . [14] have used Rituximab in a case of transformation of HL to high-grade NHL. Fourteen months later, the patient was still in complete remission including the absence of B symptoms. [14] However, the results of prospective studies in this setting are yet to be reported.

Conclusion

CD20 expression is frequent in cHL and our results are in consensus with reported literature on this subject. In these cases, LCA negativity of large cells is very useful in clinching the right diagnosis. The potential of giving anti-CD20 in cHL with CD20 co-expression for therapeutic purposes is exciting but requires further verification in robust studies.

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Figures

[Figure 1], [Figure 2], [Figure 3], [Figure 4], [Figure 5]

Tables

[Table 1]

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