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CD20 and CD10 of Chronic B-Cell Neoplasms in Correlation with Morphological Diagnosis

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Abstract

Chronic B-cell neoplasms include a number of disease entities arising from mature B lymphocytes which involve primarily the blood, bone marrow (BM) and lymphoid organs such as the lymph nodes and spleen. Chronic lymphocytic leukemia (CLL) is characterized by absolute lymphocytosis in the peripheral blood and BM. Non-Hodgkin lymphomas (NHL) also may have extensive BM and peripheral blood involvement at initial presentation like follicular lymphoma (FL), mantle cell lymphoma (MCL) and splenic marginal zone lymphoma (SMZL).

Although there are difficulties in separating CLL from some NHL, the distinction is important because prognostic and therapeutic differences exist. Immunophenotyping (IPT) has become an essential tool to confirm the diagnosis and to separate CLL from other lymphoid malignancies. Evaluate the role of immunostained CD10 and CD20 in the subclassification of chronic B-cell neoplasms and confirm the significance of these markers as a complementary test to morphological features of chronic B-cell neoplasms using BM aspirate and biopsy. BM biopsies of fifty five adult patients with CLL and leukemic phase of NHL were collected from December 2010 to April 2011; fifty of them were retrospectively while five of them were prospectively collected. Current study revealed that CLL cases (27) showed weak positive reaction with CD20 (96%) while no reaction with CD10 (0%). While NHL (28) cases showed positive reaction with CD20 (100%) & CD10 (71.5%). This study revealed significant statistical difference between morphology and IPT diagnosis at the level of P.value <0.05.

This study revealed that the CD markers has important diagnostic role in the subclassification of chronic B-cell neoplasms also revealed that the IPT technique in conjunction with morphology have more precise role than morphology alone in the diagnosis of chronic B-cell neoplasms.

Keywords: CD10, CD20, CLL, NHL

1. Introduction

Within the broad category of B-cell lymphoproliferative neoplasms a number of disease entities arising from mature B lymphocytes which involve primarily the blood, BM and other lymphoid organs such as the lymph nodes and spleen. All these disorders are classified by the World Health Organization (WHO) 2008 on the basis of their histopathological features.[1]A constant finding in all these entities is the presence in peripheral blood of leukemic cells in various degrees. Some of these conditions could be considered as CLL and others represent the leukemic phase of NHL and their recognition is important for differential diagnosis and patient management.[2]IPT markers play a key diagnostic role by enabling demonstration of the B or T cell nature of the neoplastic cells; by establishing clonality in B-cell disorders by immunoglobulin light chain restriction analysis, thus, distinguishing between neoplastic and reactive B lymphocytosis.[3]

Lymphocyte malignancies compose a wide spectrum of different morphologic and clinical syndromes. Lymphocyte neoplasms can originate from cells that are at a stage prior to T and B lymphocyte differentiation from a primitive stem cell or from cells at stages of maturation after stem cell differentiation. Thus, acute lymphocytic leukemias (ALL) arise from a primitive lymphoid stem cell that may give rise to cells with either B or T cell phenotypes. On the other hand, chronic lymphocytic leukemia arises from a more differentiated B lymphocyte progenitor and myeloma from progenitors at even later stages of B lymphocyte maturation. Variability in expression of a lymphopoietic stem cell disorder may result in the spectrum of lymphocytic diseases, such as a B lymphocyte or T lymphocyte lymphoma, and different types of diseases, such as hairy cell leukemia (HCL), prolymphocytic leukemia (PLL), natural killer cell (NK-cell), large granular lymphocytic leukemia, or plasmacytoma. [6]

WHO Classification of mature B-cell neoplasm: [11]

- Chronic lymphocytic leukemia/Small lymphocytic lymphoma
- B-cell prolymphocytic leukemia
- Hairy cell leukemia
- Lymphoplasmacytic lymphoma (Waldenström macroglobulinemia)
- Splenic marginal zone lymphoma
- Plasma cell neoplasms:
    - Plasma cell myeloma
    - Plasmacytoma

- Monoclonal immunoglobulin deposition diseases
- Heavy chain diseases

- Extranodal marginal zone B cell lymphoma, also called MALT lymphoma
- Nodal marginal zone B cell lymphoma (NMZL)
- Follicular lymphoma
- Mantle cell lymphoma
- Diffuse large B cell lymphoma
- Mediastinal (thymic) large B cell lymphoma
- Intravascular large B cell lymphoma
- Primary effusion lymphoma

Burkitt lymphoma/leukemia

The chronic/mature B-cell leukaemias include CLL, which is by far the most common, the rare B- PLL and HCL. The B- cell NHLs include FL, SMZL and MCL that most frequently affect the blood and BM and may spill over to the peripheral blood[1] . Antibodies that used in this study are CD10 and CD20[4].

2. Markers

In general, there are no surface markers that are diagnostic of malignancy in lymphocytes. Both flow cytometry or paraffin block IHC can be used to identify specific cell surface or intracellular protein expression. Flow cytometry has the advantage of simultaneous semiquantitative analysis of multiple markers.(112) However, not only it requires fresh sample for cell suspension preparation, but it is also expensive and cannot be correlated with cytoarchitectural findings. With advances in antigen retrieval techniques most antibodies can be successfully applied to paraffin blocks. Furthermore, IHC can easily identify a small population of target cells. CD20 was chosen to determine the lineage of the cells (B and T-cells). After presence of B cell is demonstrated by positive staining for CD20 (or other B cell markers). CD10 was selected for its putative role in FL. The B-cel NHLs phenotypically correspond to normal cells in the mid stages of normal differentiation. More specifically, by their expression of B-cell activation antigens, these tumors are the neoplastic counterparts of normal activated B cells[11].

2.1 CD10

Common acute lymphoblastic leukemia antigen (CALLA) is a cell-surface endopeptidase. CD10 is belonging to a family of type II transmembrane metallo proteases that also include the leucocyte antigens CD13 and CD26 as well as aminopeptidase. The gene encoding CD10 is located on chromosome 3.(12)

CD10 is a zinc-dependent enzyme and is thought to down-regulater cellular responses to peptide hormones (120) .

On lymphoid cells, CD10 is expressed on immature T-and B-precursor cells but lost as the cells reach maturation. It is re-expressed on proliferating B cells and mature neutrophiles (12) .

In lymphoid malignancy, CD10 is expressed in acute lymphoblastic leukemia(ALL) arising from precursor B cells, but also observed in a portion of T-cell ALL. Additionally, the antigen may also be expressed in FLs, Burkitt lymphoma and subsets of DLBCL. CD10 may be expressed infrequently in MCL; CD10 is expressed also in renal cell carcinoma.(12) Beside hematopoietic cells, it is also expressed in epithelial cells of the liver bile canaliculi, renal tubules, tonsil, germinal center B cells, stem cells in the BM, a subset of immature B cells in BM and neutrophiles.(13) The antibody also labels germinal center in lamina propria of colon, brush borders of the enterocytes in the small intestine, gallbladder brush border epithelium, interstitial stromal cells of the lung, Schwan nervecells and stromal cells in striated muscles, fibroblasts, trophoblast and cytotrophoblast of the placenta, prostate, stromal cells in the endometrium and breast myoepithelial cells. The staining pattern is membranous. (14).

2.1 CD20

CD20 is a transmembrane, glycosylated protein expressed on B-cells precursor and mature, but is lost following differentiation into plasma cells. The long N-and C-terminal ends of the protein are located on the cytoplasmic side of the membrane and only a minor portion of the protein is expressed on the cell surface. It is suggested that CD20 plays a direct role in regulating the transmembrane conductive Ca flux of B-cells which indicates a possible function for CD20 as a regulator of proliferation and differentiation. (125)

In normal lymphoid tissues, the antibody labeled germinal center cells, mantle zone lymphocytes, and scattered interfollicular lymphocytes but not T cells, histiocytes and plasma cells. No labeling was observed in epidermis, sebaceous glands, hair follicle and eccrine glands in the skin, follicular epithelium in the thyroid, pneumocytes and bronchial epitheliam of the lung[16].

CD20 is expressed on the great majority of mature B-cell lymphomas. This is a very useful “pan-B” cell marker. The CD20 antigen is not expressed on very immature lymphoid cells (acute

undifferentiated leukemias) but begins to be expressed on early maturational stages and then CD20 is fully expressed on mature B-cells (CLL, PLL, HCL and B-cell NHL).The staining pattern is membranous and or cytoplasmic[17].

Evaluation of CD20 expression has therapeutic importance.A humanized monoclonal antibody (Rituximab) against CD20 is now available for treatment of B-cell lymphomas expressing this molecule. Thus, CD20 expression is used as a criterion for administering Rituximab. Following treatment of FL with anti -CD20 monoclonal antibodies, the trephine biopsy sections may show disappearance of the B-cell infiltrate but persistence of reactive T cells). In some patients, there is a change of IPT shortly after such immunotherapy with B cells failing to express CD20; this may be persistent for several months and does not correlate with a failure of response[18]

The aim of current study was to evaluate the role of immunostained CD10 and CD20 in the subclassification of chronic B-cell neoplasms and confirm the significance of these markers as a complementary test to morphological features of chronic B-cell neoplasms using BM aspirate and biopsy.

3. Materials

BM biopsies of fifty five adult patients with CLL and leukemic phase of NHL were collected from December 2010 to April 2011 with age range of 28-80 years; fifty of them were conducted retrospectively from archive files of the Department of Hematology of the Medical City Teaching Laboratories while five of them were conducted prospectively from private laboratories. For each case one section was stained with hematoxylin and eosin and two other sections were stained immunohistochemically (IHC) for CD10 and CD20.

Primary antibody kit as shown in table 2.13.

Table 1. Primary antibody kit used in this study

Primary antibody	Source	Type	Code number
CD10	Dako Cytomation	Monoclonal Mouse Anti-Human CD10	M7308
CD20	Dako Cytomation	Monoclonal Mouse Anti-Human CD20	M0755

Scoring systems for markers: The scoring system for all markers was scored positive if 25% or more of the cells within an aggregate showed cellular membrane and/or cytoplasmic staining pattern[5]

Staining patterns: The cellular staining pattern is membranous and/or cytoplasmic for CD10[7] and CD20[8].

4. Results

Current study revealed that CLL cases (27) showed positive IPT reaction with CD20 (96%) while no reaction with CD10 (0%).Moreover, NHL (28) cases showed positive reaction with CD20 (100%), CD10 (71.5%).

Furthermore, FL showed positive reaction with CD10 and CD20 in 100% of cases while MCL showed 100% positive reaction with CD20 and no reaction with CD10. So significant statistical difference between morphology and IPT diagnosis at the level of P.value <0.05.

Table 2. IHC findings in CLL patients

CD10		CD20	
positive	negative	positive	negative
0%	100%	96%	3.7%
0/27	27/27	26/27	1/27

Table 3. IHC findings in NHL patients

CD10		CD20	
positive	negative	positive	negative
71.%	28.5%	100%	0%
20/28	8/28	28/28	0/28

Table 4. subtypes of NHL diagnosed by IPT

FL	MCL	Other Low grade B-cell NHL
20/28 (71.5%)	4/28 (14%)	4/28 (14%)

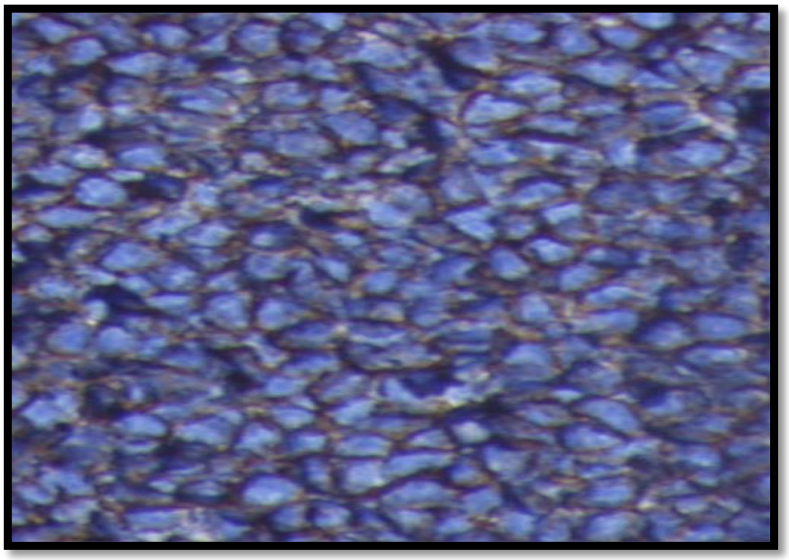

Figure 1. Photomicrograph shows brown membranous and cytoplasmic IHC stain with CD20 in bone marrow tissue of NHL patient (x40)

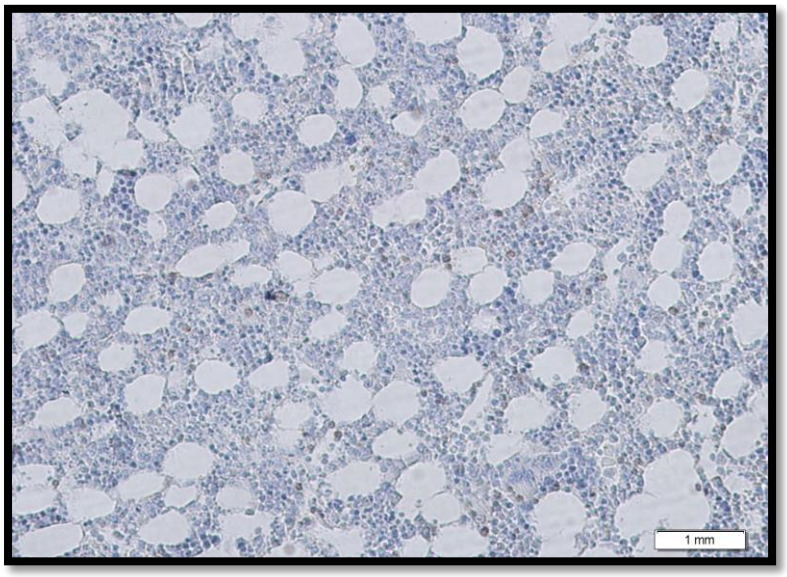

Figure 2. Photomicrograph shows no brown membranous or cytoplasmic IHC stain with CD20 in BM tissue of CLL patient (x10)

4. Discussion

The chronic/mature B-cell neoplasms include CLL, which is by far the most common, the rare B-PLL and HCL. The B-cell NHLs include FL, SMZL, and MCL that most frequently exhibit circulating lymphoma cell(1) .

IHC is useful in distinguishing between reactive and neoplastic lymphoid infiltration. The availability of a broad panel of antibodies suitable for paraffin-embedded tissues enables us to perform complete IPT on BM trephines and allows for classification of lymphoid infiltrates.[4]

CD20: In this study CD20 present in more than 25 % of cells within aggregate as cellular membrane and/or cytoplasmic stain in 26 cases of CLL so it is 96% positive in CLL group.

So twenty seven cases of CLL showed CD20 positive membranous and/or cytoplasmic stain reaction and negative reaction with CD10 as agreement with most studies (16, 17), so only one case of CLL was negative for CD20.

Twenty eight cases of NHL showed CD20 positive membranous & or cytoplasmic reaction and majority (71.5%) showed CD10 positive membranous reaction, this agree with other workers(20 ,19) .

CD10: twenty cases of NHL showed CD10 positive membranous staining reaction were diagnosed as FL [5].

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