Original paper

Immunophenotyping of Mature B-Cell Neoplasms in Correlation with Morphological Diagnosis

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Abstract

Background: Mature 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.
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.
Objectives: To express the difference between the IPT& morphology with morphology alone to reach definite diagnosis in mature B- cell neoplasm.
Methods: 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.
Results: In this study, Twenty six out of 29 CLL patients were confirmed as CLL by IPT while the rest three cases diagnosed as NHL rather than CLL and 25 out of 26 NHL cases were confirmed by IPT and only one case diagnosed as CLL.
Conclusions: This study revealed that the IPT has important diagnostic role in the subclassification of mature B-cell neoplasms also revealed that the IPT technique in conjunction with morphology have more precise role than morphology alone in the diagnosis of mature B-cell neoplasms.
Key words: CD5, CD10, CD20, CD23, Light chains, CLL, NHL.

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)
Antibodies that were used in this study are CD5, CD10, CD20, CD23 and Kappa and lambda Light chains. (4)
The aim of current study was to express the difference between the IPT & morphology with morphology alone to reach definite diagnosis in mature B-cell neoplasms.

Materials & methods

BM biopsies of fifty five adult patients with CLL and leukemic phase of NHL

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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 six other sections were stained immunohistochemically (IHC) for CD5, CD10, CD20, CD23 and light chains (Kappa and Lambda) .

Primary antibody kit as shown in table 1.

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 CD5 [6], CD10 [7], CD20 [8], kappa and Lambda [9] and display membrane staining for CD23 [10].

Results

In this study, 29 cases of CLL patients were initially diagnosed by morphology while by IPT, 26 were confirmed as CLL and the another 3 cases diagnosed as NHL, while 26 cases of NHL were diagnosed by morphology but by IPT, 25 cases were consisting with NHL and one case diagnosed as CLL so significant statistical difference between morphology and IPT diagnosis at the level of P.value <0.05.

Current study revealed that CLL cases (27) showed positive IPT reaction with CD5 (100%), CD23 (100%), CD20 (96%) while no reaction with CD10 (0%) and either with Kappa (52%) or Lambda (48%).

Moreover, NHL (28) cases showed positive reaction with CD20 (100%), CD10 (71.5%), low reaction with CD5 (14%), either with Kappa (46.5%) or Lambda (53.5%) and no reaction with CD23.

According to IPT findings, the NHL cases can be classified roughly into FL (Follicular lymphoma) in 20/28 (71.5%), MCL (Mantel cell lymphoma) in 4/28 (14%) and other B-cell NHL 4/28 (14%).

Furthermore, FL showed positive reaction with CD10 and CD20 in 100% of cases and no reaction with CD5 and CD23 while MCL showed 100% positive reaction with CD5 and CD20 and no reaction with CD23 and CD10.

Table 1. Primary antibody kit used in this study:

Primary antibody	Source	Type	Code number
CD5	Dako Cytomation	Monoclonal Mouse Anti-Human CD5	M7194
CD10	Dako Cytomation	Monoclonal Mouse Anti-Human CD10	M7308
CD20	Dako Cytomation	Monoclonal Mouse Anti-Human CD20	M0755
CD23	Dako Cytomation	Monoclonal Rabbit Anti-Human CD23	M7306
Kappa	Dako Cytomation	Polyclonal Rabbit Anti-Human Kappa light chain	A0191
Lambda	Dako Cytomation	Polyclonal Rabbit Anti-Human Lambda light chain	A0193

Table 2. IHC findings in CLL patients

CD5		CD23		CD10		CD20		kappa		Lambda	
positive	negative	positive	negative	Positive	negative	positive	negative	positive	negative	positive	negative
100%	0%	100%	0%	0%	100%	96%	3.7%	52%	48%	48%	52%
27/27	0/27	27/27	0/27	0/27	27/27	26/27	1/27	14/27	13/27	13/27	14/27

Table 3. IHC findings in NHL patients:

CD5		CD23		CD10		CD20		kappa		Lambda	
positive	negative	positive	negative	positive	negative	positive	negative	positive	negative	positive	negative
14%	86%	0%	100%	71.%	28.5%	100%	0%	46.5%	53.5%	53.5%	46.5%
4/28	24/28	0/28	28/28	20/28	8/28	28/28	0/28	13/28	15/28	15/28	13/28

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%)

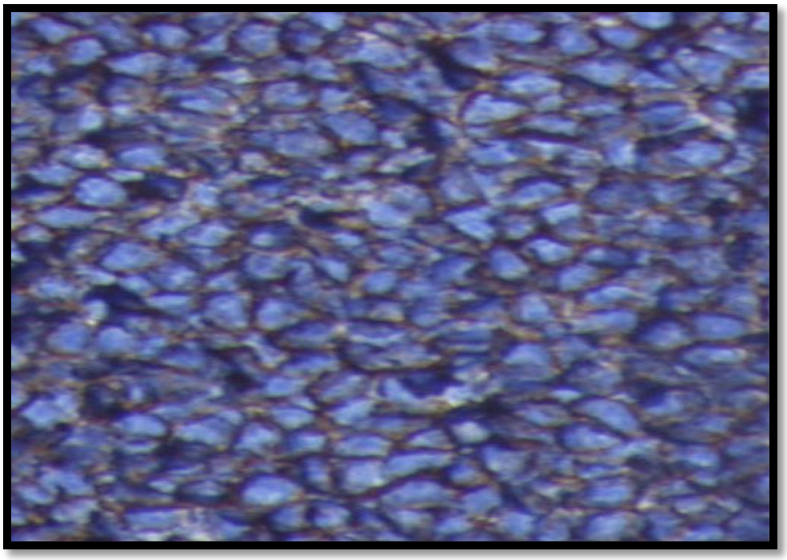

Figure 1. Photomicrograph shows brown membranous and cytoplasmic IHC stain with CD20 in bone marrow tissue of NHL patient (x40)

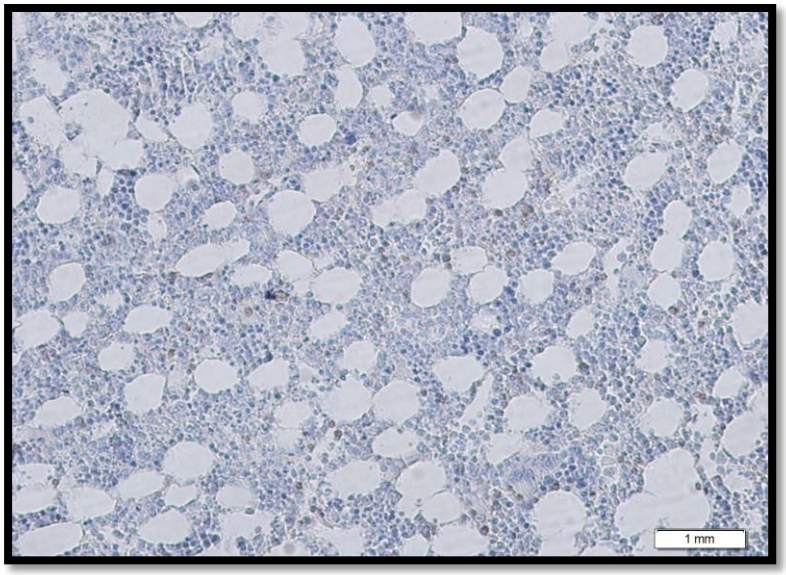

Figure 2. Photomicrograph shows no brown membranous or cytoplasmic IHC stain with CD20 in BM tissue of CLL patient (x10)

Discussion

The 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. [14]

CLL markers:

CD 5: In this study CD5 present in more than 25% of cells within aggregate as cellular membrane and/or cytoplasmic stain in all cases of CLL and MCL so it is 100% positive in CLL group and MCL group. Similar results were reported by D 'Arena who had use IPT study of CD5 expression in BM of CLL cases by flowcytometry technique. [15, 16]

CD 23: In this study CD23 present is more than 25 % of cells with in aggregate as cellular membrane and/or cytoplasmic stain in all cases of CLL so it is 100% positive in CLL group. Similar results were reported by V Deneys who had use IPT study of CD23 expression in BM of CLL cases by flowcytometry technique. [16]

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 CD5 and CD23 (100%) positive membranous and/or cytoplasmic stain reaction and 96% of cases showed CD20 positive membranous and/or cytoplasmic stain reaction and all showed clonality by Ig light chain restriction and negative reaction with CD10 as agreement with most studies [16, 17], so only one case of CLL was negative for CD20.

NHL markers: Twenty eight cases of NHL showed CD20 positive membranous & or cytoplasmic reaction and majority (71.5%) showed CD10 positive membranous reaction while (14%) showed positive membranous & or cytoplasmic reaction with CD5, no reaction with CD23 and all showed monoclonality by Ig light chain restriction with either Kappa or Lambda, this agree with other workers. [19, 20]

CD10: According to IPT, twenty cases of NHL showed CD10 positive membranous staining reaction were diagnosed as FL [5] and all showed monoclonality by Ig light chain restriction and no reaction with CD23 and CD5 similar with other workers. [19, 20]

There were three patients initially diagnosed as CLL but subsequently by IPT consistent with FL with leukemic presentation because had CD5, CD23 negative and CD10, CD20 positive and had BM biopsies in which the morphology of the lymphoid cell did not distinguish between CLL and NHL. The lymphoid infiltrates in the trephine biopsies were interstitial, diffuse and mixed these infiltrates composed of small lymphocytes with condensed chromatin. Those patients presented with absolute lymphocytosis with WBC were $143.4x10^9$/L, $95.8x10^9$/L and $68.3x10^9$/L respectively. The differential showed 94%, 85% and 90% of ANC with circulating lymphocytes are typically small with scant cytoplasm and cleaved nuclei but included 10% larger cells with ample cytoplasm and central nucleoli consistent with prolymphocytes and few smudge cells are seen in all three cases.

Also there was one case in which initially diagnosed as NHL (FL) while by IPT consistent with CLL because had CD10 negative and CD5, CD23 positive which is unusual for NHL and the pattern of BM infiltration was focal non-paratrabecular, this infiltrate composed of small lymphocytes with condensed chromatin. The WBC count was $65x10^9$/L and

peripheral blood lymphocyte count was 62% of ANC. The peripheral blood showed a uniform population of small lymphocytes with condensed chromatin and scant cytoplasm. Only rare lymphocytes showed slight nuclear indentations and few smudge cells are seen.

In this study, it was important to use CD79b and FMC antibodies to perform complete distinction between NHL and CLL which is typically FMC7 negative but these two markers cannot be performed by IHC and only done by flowcytometry. Also it was necessary to do double check of B or T cells by CD3 but the BM blocks were very tiny tissues and additional antibodies were difficult to be added.

We have shown that no single antibody that we tested in this study reliably distinguishes Chronic B- cell neoplasms involving the BM, so a panel of antibodies is recommended for precise IPT diagnosis.

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