Immunophenotyping of Bone Marrow Biopsies in 26 Patients with Non-Hodgkin's Lymphoma, Using Anti-CD3, CD8, CD19 and CD20 Monoclonal Antibodies

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ABSTRACT:

BACKGROUND:

Immunophenotyping with monoclonal antibodies (MoAbs) directed against lymphoid-associated antigens, immunohistochemical staining on paraffin-embedded BM biopsy material, and molecular studies of Ig genes/T-cell receptor genes or lymphoma-associated gene translocations should be used in the global approach to the patient with malignant lymphoma.

OBJECTIVE:

1. To determine the subtypes of non-Hodgkin lymphomas (B- or T cell) in the bone marrow using anti-CD3, CD8 monoclonal antibodies for T-cell and anti-CD19 and CD20 for B-cells.
2. Correlation of the subtypes of NHL (B- or T cell) with the morphology and pattern of bone marrow infiltration.

PATIENTS, MATERIALS AND METHODS:

A retrospective study, done in Al-Kadhymia teaching hospital during the period from 1/10/2010 to1/2/2011.The study consisted 26 adult patients, who were diagnosed as Non-Hodgkin lymphomas by undergoing a BM biopsy. Immunohistochemical staining of the paraffin-embedded sections of BM trephine biopsies was performed in all cases and used standard techniques with monoclonal anti-CD8, CD20 and dual immunofluorscence-labelled CD3, and CD19 antibodies and also all stained with Hematoxylin and Eosin (H&E) for morphologic assessment.

RESULTS:

The 26 cases of NHL comprised of 14 male (54%) and 12 female patients (46%) . The median age was (57.32) year ranged from 27-85 years. There were 23 cases of B-cell cases (88.5%) and 3 cases of T-cell lineage (11.5%) of all the cases. Among all the B-cell lymphomas, 15 cases showed interstitial infiltration in the bone marrow, while among the T-cell lymphoma two cases showed diffuse infiltration.

CONCLUSION:

1. In these 26 cases NHL patients with marrow involvement, B cell phenotype comprised 88% of cases.
2. B-cell NHLs had predominance of interstitial infiltration in bone marrow biopsies in comparison with the T-cell lymphoma, in which diffuse infiltration was predominant.

KEYWORDS: non-hodgkin's lymphoma, immunophenotyping , monoclonal antibodies.

INTRODUCTION:

Malignant lymphoma is the most common hematologic malignancy encountered in the Western world.[1,2]. Non-Hodgkins Lymphoma is the 4th commonest cancer in Iraq in 1995-1999. [3] The laboratory evaluation of patients with malignant lymphoma remains centered on 4 primary aspects: [1] recognition and diagnosis of disease; [2] appropriate classification; [3] providing information regarding disease stage; and [4] providing prognostic indications that predict the risk of death from disease. [4,5]

However, obtaining a bone marrow (BM) biopsy has remained an important part of evaluating these patients. BM involvement by lymphoma has traditionally been detected by morphologic assessment of biopsy specimens. Compared with unilateral BM biopsies, the evaluation of bilateral trephine specimens apparently increases the rate of detection of lymphoma by 10% to 20%, although studies have not precisely controlled for the size of the specimen examined. [6]

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Immunophenotyping with monoclonal antibodies (MoAbs) directed against lymphoid-associated antigens, immunohistochemical staining on paraffin-embedded BM biopsy material, and molecular studies of Ig genes/T-cell receptor genes or lymphoma-associated gene translocations should be used in the global approach to the patient with malignant lymphoma.[7,8]

AIM OF THE STUDY:

1-To determine the subtypes of non-Hodgkin lymphomas (B- or T cell) in the bone marrow using anti-CD3, CD8 monoclonal antibodies for T-cell and anti-CD19 and CD20 for B-cells.

2-Correlation of the subtypes of NHL (B- or T cell) with the morphology and pattern of bone marrow infiltration.

PATIENTS, MATERIALS AND METHODS:

A retrospective study, done in Al-Kadhymia teaching hospital during the period from 1/10/2010 to1/2/2011.The study consisted of 26 adult patients who had undergone a BM biopsy for staging, preceded by concurrent lymph node or other tissue biopsy that was diagnosed as non-Hodgkin lymphoma, with positive infiltration of the bone marrow were identified for this study.

The samples were collected from medical city teaching laboratories (15 cases) and Al-kadhymia teaching laboratories (11 cases). BM trephine biopsies, performed under local anesthesia, were obtained in all cases using the conventional technique with Jamshidi needle from the posterior superior iliac spine, fixed in 10% buffered formalin solution, decalcified using 10% formal-formic acid for 4-6 hours, followed by paraffin-embedding. Five sections were cut for each specimen with 4-6 micron thickness levels, and one stained with hematoxylin and eosin (H&E) for morphologic assessment of malignant lymphoma.

Immunohistochemical staining of the paraffin-embedded BM biopsies was performed in all cases and used standard techniques with monoclonal anti-CD8, CD20 and dual immunofluorescence-labelled CD3, and CD19 antibodies. Positive CD19 and CD20 staining defined B-NHL while positive CD3 and CD8 defined T-NHL.

Control samples of normal bone marrow and lymph node biopsies were selected and stained with and without the mentioned monoclonal antibodies as positive and negative controls.

Immunoreagents and immunocytochemical procedures

Three thin paraffin-embedded sections (4 µm thick) of bone marrow tissue section were mounted on poly-l-lysin-couted (positively charged) slides for the immunocytological characterization in these tissue sections. Staining was done according to the instructions given by manufacturing company AbD Soretec for dual immunofluoresence and Dakocytomation Denmark company for CD20, CD8.

Immunohistochemistry with anti- CD20 antibodies

Principle

Specimen preparation and staining procedure reference is Dakocytomation, monoclonal mouse, anti-human CD20cy, clonal L26, Code No./Code/Code-Nr.M0755, Edition/ Ausgabe 18.12.02.

Immunohistochemistry with anti- CD8 antibodies

Principle

Specimen preparation and staining procedure reference is Dakocytomation, monoclonal mouse, anti-human CD8, clone CD8/144B, Code No./Code/Code-Nr.M0703, Edition/ Ausgabe 17.12.02.

Direct dual-Immunofluorescence procedure:

Specimen preparation and staining procedure reference is AbD SeroTec, monoclonal mouse, anti-human CD3,: FITC/CD19:RFE.

Statistical analysis:

Numerical data were recorded as mean, standard error and standard deviation, independent sample t-test was used to compare the mean values. Categorical data were expressed as frequency and percentage, chi-square was used to compare between different study groups and p-value ≤ 0.05 was considered indicative of statistically significant difference.

RESULTS:

The 26 cases of NHL comprised of 14 male (54%) and 12 female patients (46%). The Median age was 57.32 year ranged from 27-85 years. The predominant histological pattern of involvement by lymphoma atous infiltrate was interstitial 15 cases (57.6%), followed by mixed (focal, interstitial) 4 cases (15.3%), random focal infiltration 3 cases (11.5%), diffuse 3 cases (11.4%) and the least was the nodular pattern, only one case (3.8%). As shown in Table 1.

Table 1: Frequency of different patterns of bone marrow infiltrations in NHL.

Bone marrow infiltration type	Frequency	Percent
Diffuse infiltration	3	11.5
focal infiltration	3	11.5
Interstitial infiltration	15	57.6
Mixed infiltration	4	15.3
Nodular infiltration	1	3.8
Total	26	100

All the 23 cases B-cell lymphomas were both CD19 and CD20 positive, while all the 3 cases of T-cell lymphomas were CD3cyt and CD8 positive except one case which was CD8 negative.

Table 2: Frequency of B-cell and T-cell in NHL cases.

		Frequency	Percent
Type of lymphoma	B cell	23	88.5%
	T cell	3	11.5%
	Total	26	100%

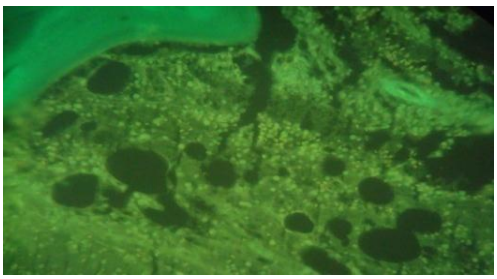

Figure 1: : Bone marrow biopsy of NHL showing, positive immuno- fluorscence stain reaction to Rhoudamin anti-CD19 antibody power x100.

Table 3: Distribution of bone marrow infiltration pattern in B and T-cell NHL.

Bone marrow infiltration	Type of lymphoma				
	B cell N=23		T cell N=3		
	Count	Percentage	Count	Percentage	N=26
Diffuse infiltration	1	3.85%	2	7.69%	3
focal infiltration	3	11.54%	0	0.00%	3
Interstitial infiltration	15	57.69%	0	0.00%	15
Mixed infiltration	3	11.54%	1	3.85%	4
Nodular infiltration	1	3.85%	0	0.00%	1
Total	23	88.46%	3	11.54%	26

Chi-square p value=0.062.

DISCUSSION:

In the current study, the bone marrow infiltration by B-cell NHL is more common than that of the T-cell in a percent of 88.5%, compared to 11.5%. All the cases of NHL selected in our study, had been already infiltrated to the bone marrow, and because the prevalence of B-cell type is more common than that of T-cell lymphomas. So it was logical to find more B-cell infiltration than

T-cell type.[9] The number of T-cell cases in our study was so small (just 3 cases) that we couldn`t depend on it, so we need large number of T-cell cases to get better results

The 3 cases of T-cell lymphomas were CD3cyt and CD8 positive except one case which was CD8 negative and that might be of helper CD4 cell type, and incidence of the pattern of T-cell bone marrow involvement was diffuse type (two out of 3 cases).

The predominant histological pattern of involvement by lymphoma tous infiltrate was interstitial, followed by mixed (focal, interstitial) cases.

CONCLUSION:

1. In these 26 cases NHL patients with marrow involvement, B cell phenotype comprised 88% of cases.
2. B-cell NHLs had predominance of interstitial infilteration in bone marrow biopsies in comparison with the T-cell lymphoma, in which diffuse infiltration was predominant.

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