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Original Research Article

Role of Flow Cytometry in Diagnosis of Non-Hodgkin's Lymphoma: A Study at RIMS, Ranchi, India

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Abstract:

Purpose: Non-Hodgkin's lymphomas (NHL) are cancers of mature B, T and NK cells. Diagnosis of NHL is based on immunophenotyping by immunohistochemistry of excised lymph node or flow cytometric immunophenotyping. FNA cytology with immunolabelled flow cytometry can in some circumstance serve as a replacement of open biopsy and conventional histology and immunohistochemistry.

Method: The present study consisted of 52 patients suffering from lymphoreticular malignancies who attended and/or were admitted in the Rajendra Institute of Medical Sciences, Ranchi, during the period from March 2020 to August 2021.

Results: Out of 54 cases suspicious of Lymphoreticular malignancy, 2 were found to be non-lymphoid origin by flow cytometry of fine needle aspirate and sample collection was inadequate in 3 cases (5.5%). On Immunophenotyping, 44 cases (88%) were found to be B-NHL and 5 cases (10%) were T-NHL. Furthermore, it was sublassified as DLBCL 16 cases (32.6%), FL 6 cases (12.2%), MZBCL 3 cases (6.1%), CLL/SLL 5 cases (10.2%), MCL 2 cases (4.08%), Unclassified B-NHL 12 cases (20.3%) and Mature T cell NHL 5 cases (10.2%).

Conclusion: Flowcytometry can be a great tool in diagnosis and classification of NHL and can be used alternatively to conventional excisional Lymph node biopsy and immunohisto-chemistry.

Keywords: Flow cytometry, immunophenotyping, Non Hodgkin's Lymphoma, FNA cytology

Introduction

Non-Hodgkin's lymphomas (NHL) are cancers of mature B, T and NK cells. NHL can be classified as either a mature B-NHL or a mature T/NK-NHL depending on whether the malignant lymphocyte is a B, T or NK cell respectively. The modern classification of NHL according to World Health Oraganization/ Revised European American Lymphoma (WHO/REAL) is based on morphology, immunology, cytogenetics and molecular studies.8

Diagnosis of NHL is based on clinical features, radiological examinations, fine needle aspiration cytology and immunophenotyping by immunohistochemistry of excised lymph node or flow cytometric

immunophenotyping. Immunophenotyping helps in sub-classification of NHL which determines management and prognosis of the disease. Currently many centers employs the technique of immunohistochemistry of excised lymph nodes to arrive at a conclusive diagnosis and sub-classification of nodal lymphoma. But it is being largely replaced by immunoflowcytometry. It is accepted that FNA cytology with immunolabelled flow cytometry can in some circumstance serve as a replacement of open biopsy and conventional histology and immunohistochemistry.9-14

Flow cytomtry is a technique in which a fluorescent dye which is coupled to a monoclonal antibody binds to those cells coated with the antigen for which the antibody is specific. So antigenic characteristics of a malignant cell of interest can be determined by simultaneous binding of multiple monoclonal antibodies coupled with different fluorochromes. The stream of cells is passed through a laser beam and light scattering by the cells is analyzed by flow cytometer software.

Material and Methods

The present study consisted of 52 patients suffering from lymphoreticular malignancies who attended and/or were admitted in the Rajendra Institute of Medical Sciences, Ranchi, during the period from March 2020 to August 2021. The study was conducted in the department of Pathology of Rajendra Institute of Medical Sciences, Ranchi (Institutional Ethics Committee Letter no. 203 RIMS, Ranchi, Dated 21/12/2019). Proper clinical history was taken followed by Fine needle aspiration cytology of enlarged lymph nodes. Based on the microscopic findings of FNAC, those cases diagnosed as lymphoreticular malignancy were considered for immunophenotyping by flow cytometry. The fine needle aspirate of the involved lymph nodes was processed into single cell suspension by addition of phosphate buffer saline, disintegration of tissue along with RBC lysis, washing with phosphate buffer saline and centrifugation. Single cell suspension thus formed was concentrated to at least 10000 cells/microliter. Immunohenotyping was done using BD biosciences 6 color Flow-cytometer. Table 1 shows Chronic Lymphoproliferative Diseases (CLPD) panel where Tube1 has CD19 only, tube 2 and 3 has B-cell markers and tube 4 has T-cell markers.

Results

Out of a total of 54 cases selected for the study 2 cases were CD45-ve on flow-cytometric immunophenotyping, which imply they were not NHL as suspected in FNAC. 3 cases could not be studied in flow cytometry due to insufficient sample collection during FNAC and failure of single cell suspension preparation. Out of 49 cases, 44 (90%) were found to be B-NHL and 5 (10%) were T-NHL. 12 cases (24%) of B-NHL could not be sub-classified based on present CLPD panel of monoclonal antibodies.

Table 2 shows incidence of B-NHL is higher than T-NHL.
Table 3 shows the incidence of various subtypes of NHL.
Table 4 shows adequacy of sample collection in various studies done worldwide.
Table 5 shows various sites of lymph node enlargement in NHL.
Figure 1 shows that incidence of B-NHL is more than that of T-NHL.
Figure 2 shows CD19 gated lymphoid cells with positivity for CD10, CD20; negative for CD5 T-NHL.
Figure 3 shows CD19 gated lymphoid cells with positivity for CD200 and CD23; negative for FMC7 in SLL/CLL.
Figure 4 showing CD19 gated cells with positivity for CD5; negative for CD10 and CD20 (original image).
Figure 5 shows CD3 gated lymphoid cells with positivity for CD45, CD7 and negative for CD4, CD8 indicating T-NHL.

Table 1: Table showing the CD markers used in different tubes for the purpose of immunophenotyping (original table)

Tubes	FITC	PE	PerCP5.5	PE-Cy7	APC	APC-H7
Tube 1 (unstained)				CD19		
Tube 2 (B-CLPD1)	Lambda	Kappa	CD5	CD19	CD10	CD20
Tube 3 (B-CLPD2)	FMC7	CD200	CD38	CD19	CD23	CD45
Tube 4 (T-CLPD)	CD8	CD5	CD3	CD4	CD7	CD45

Table 2: Table showing the incidence of various NHL (original table)

Type of NHL	Frequency	Percentage
B-NHL	44	89.8
T-NHL	5	10.2

Table 3: Table showing the incidence of various subtypes of NHL (original table)

Subtypes of NHL	Frequency	Percentage
DLBCL	16	32.6
FL	6	12.2
MZBCL	3	6.1
CLL/SLL	5	10.2
MCL	2	4.08
Unclassified B-NHL	12	20.3
Mature T cell NHL	5	10.2
Total	49	100

Table 4: Table showing adequacy of sample collection in various studies done worldwide (reference of data cited)

Authors	Year	Total cases	Inadequate sampling
Paul et al[8]	2014	14	20 (14.2%)
Mayall et al[14]	1999	73	9 (12%)
Young et al[17]	1998	107	5 (4.7%)
Demurtas et al[20]	2013	1792	19 (1%)
Dong et al[12]	2001	139	34 (24%)
Present study	2021	54	3 (5.5%)

Table 5: Table showing the site of lymph node enlargement NHL cases (original table)

Group of Lymph nodes involved	Cervical	Axillary	Inguinal	Submandibular	Parotid	Supraclavicular	Others
Total no. of cases	24 (49%)	12 (24%)	17 (35%)	3 (6%)	4 (8%)	7 (14%)	4 (8%)

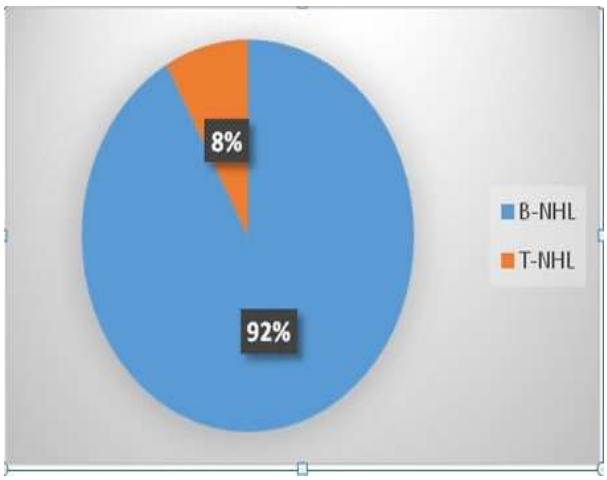

Figure 1: Pie chart showing patients with B-NHL and T-NHL (original image)

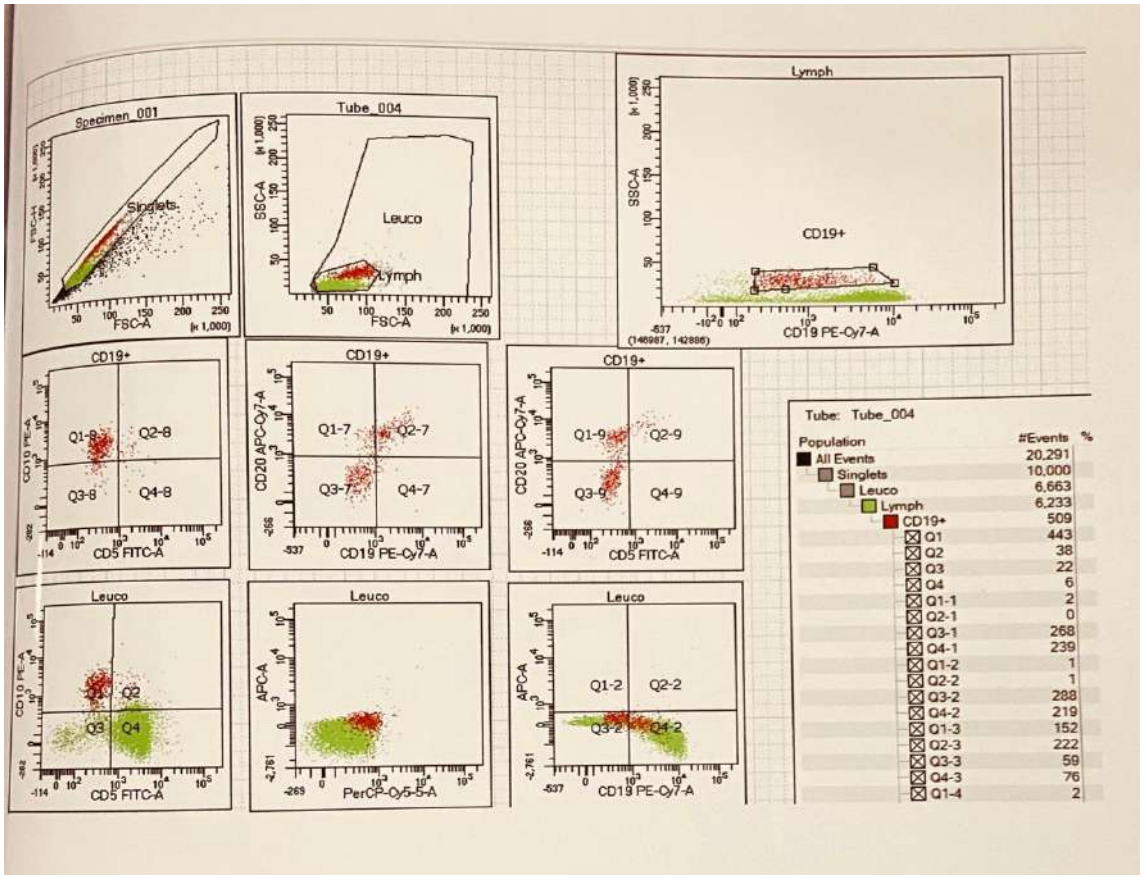

Figure 2: Graphs showing CD19 gated lymphoid cells with positivity for CD10, CD20; negative for CD5 (original image)

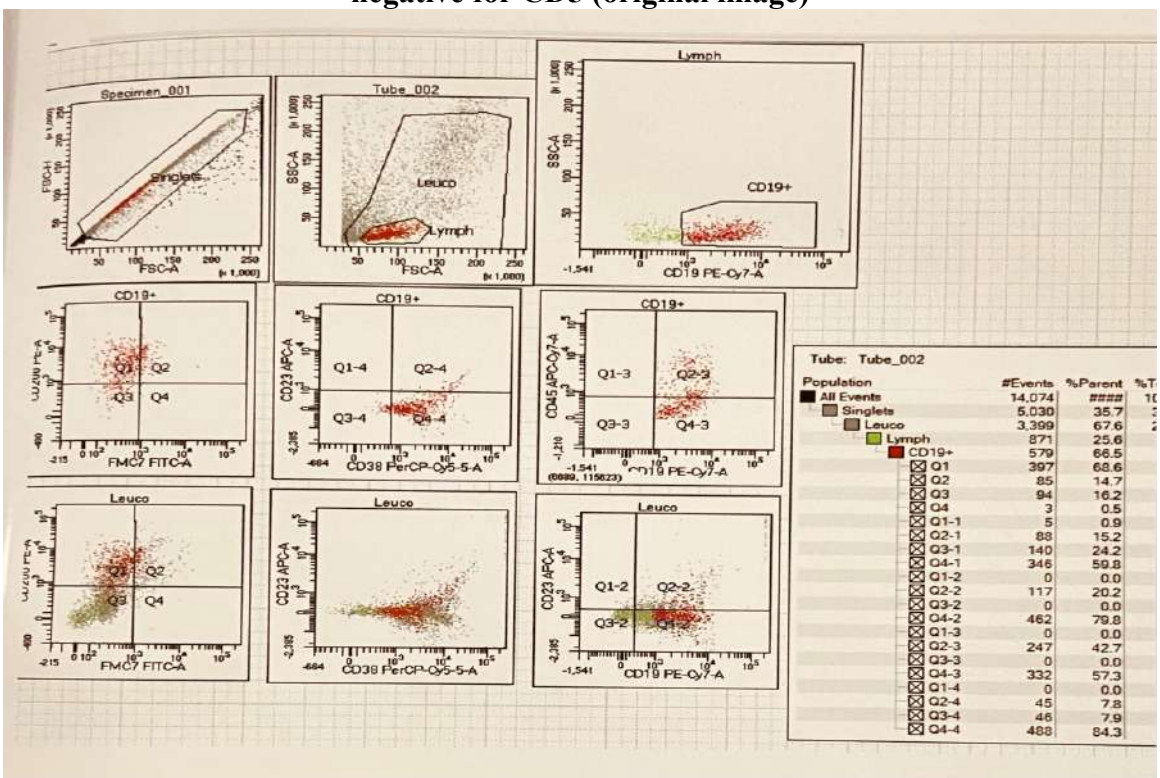

Figure 3: Graphs showing CD19 gated lymphoid cells with positivity for CD200 and CD23; negative for FMC7 in SLL/CLL (original image)

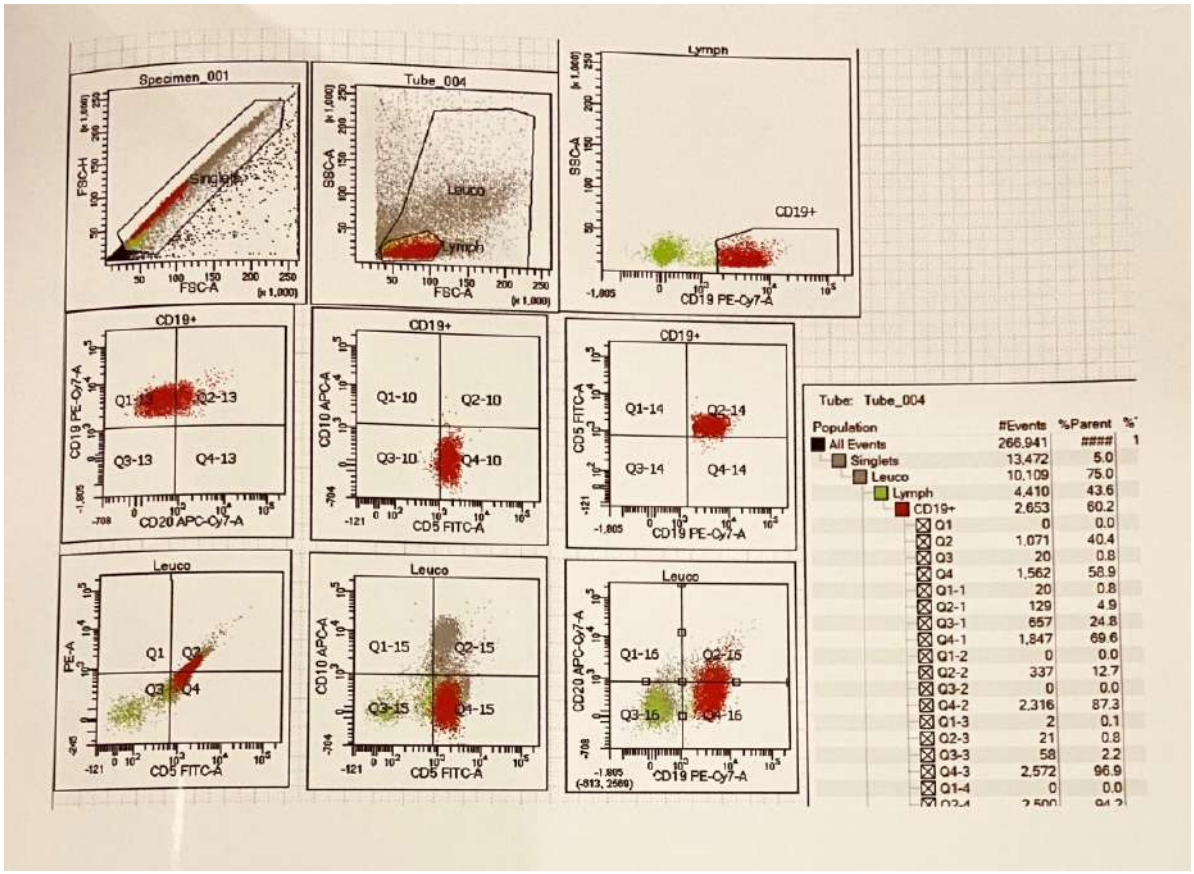

Figure 4: Graphs showing CD19 gated cells with positivity for CD5; negative for CD10 and CD20 (original image)

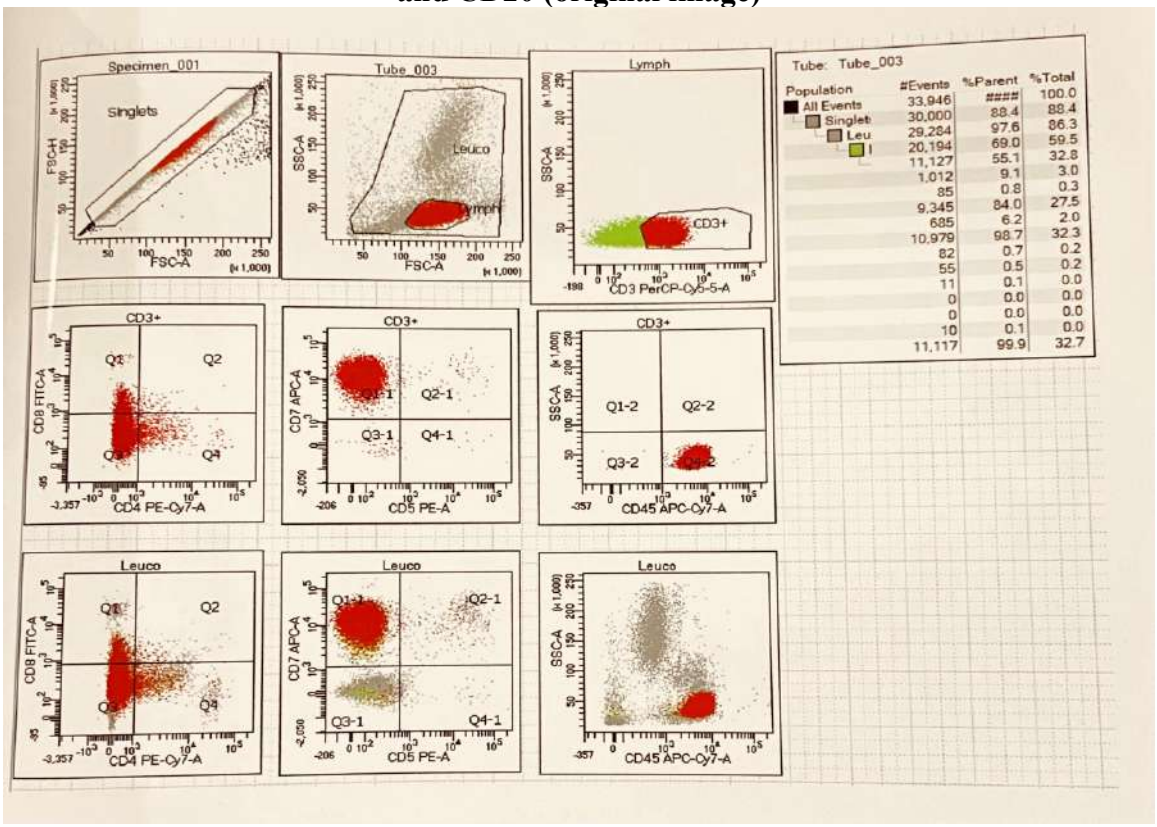

Figure 5: Graphs showing CD3 gated lymphoid cells with positivity for CD45, CD7 and negative for CD4, CD8 in T-NHL (original image)

Discussion

The incidence of B-NHL (90%) was found to be more than T-NHL (10%).

2 cases presumed to be NHL by conventional FNA cytology were ruled out by flow-cytometric immunophenotyping as they were CD45 negative. Most common group of lymph nodes involved were cervical, axillary, Inguinal, submandibular, parotid and supraclavicular.

Sample collection and processing were adequate for 94.5% cases which was better than most of the studies done worldwide.

32 (76%) cases of B-NHL were successfully sub-classified based on flow cytometric immunophenotyping and 12 (24%) cases remained unclassified due to limitation of CLPD panel used. The limitation of this study is that the diagnosis was not confirmed by lymph node excision biopsy and immunohistochemistry so a comparison between the two modalities of immunophenotyping could be done.

Conclusion and Take Home Message

As immunophenotyping was feasible from lymph node fine needle aspirate, Flow cytometry could be used as alternate diagnostic modality for solid non-Hodgkin's lymphomas also which is hitherto used only for leukemias.

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