Immunophenotyping analysis of lymph node biopsies by flow cytometry

Raja-Zahratul A. Raja-Sabudin, MBBS, MPath,
Azura A. Hamid, MD, MPATH,
Nurasyikin Yusof, MBChB, FRCPA,
Hafiza Alauddin, MBBS, MPath, *Suria A. Aziz,* MBBS, MPath,
Sivagengei Kulaveerasingam, Dip MLT,
Nozi M. Zin, Dip MLT, *Siti-Aishah M. Ali,* MBBCh, DCP,
Rohaizak Muhammad, MBChB, FRCS, *Srijit Das,* MBBS, MS,
Ainoon Othman, MBBS, Dr Med Sc,
Noor H. Hussin, MBBCh, Dr Med Sc.

The conventional laboratory diagnosis of lymphoma using tissue biopsies is based on histology diagnosis and immunohistochemistry. However, this method is tedious and involves multiple steps and procedures. In developed countries, immunophenotyping by flow cytometry is readily performed on tissue biopsies such as lymph nodes for diagnostics purposes. However, the scenario is different in Malaysia where flow cytometric analyses of body tissues are not readily available. Fine-needle aspiration of lymph node tissues combined with flow cytometric immunophenotyping (FCI) has been reported to be successful in evaluating sites for lymphomatous involvement in 75-90% of cases.[1,2] Dunphy[3] described the contribution of FCI in a series of 373 tissue specimens (278 lymph nodes and 95 extra-nodal tissue) from patients with suspected lymphoma. The results showed that the FCI data was consistent with the final tissue histological diagnosis in the majority (94%) of the tissue samples. Ravoet et al[4] evaluated the contribution of flow cytometry to the diagnosis of malignant and non malignant conditions in lymph node biopsies in 116 samples. The results showed that flow cytometric analyses of the lymph node biopsies were in agreement with tissue histology in 102 cases (87.9%). In view of the facts mentioned above, we embarked on this study to assess the role of FCI on lymph node biopsies in providing a diagnosis of hematological malignancies, especially the non Hodgkin lymphoma (NHL), in University Kebangsaan Malaysia Medical Centre (UKMMC) setting.

This was a descriptive cross-sectional study of lymph node specimens from patients who were subjected to diagnostic lymph node biopsies. All lymph node specimens were processed in the Hematology and Histopathology Laboratories, Diagnostic Laboratory Services Department, UKMMC, Kuala Lumpur, Malaysia, between November 2007 and October 2008. Informed consent was taken from each patient involved in this study. Patients with lymphadenopathies or with suspected hemato-lymphoid malignancies who were subjected to excisional lymph node biopsies for diagnostic purposes in UKMMC were enrolled in this study. The lymph node tissues, which were excised from patient, were cut into halves. One portion was collected into a container containing RPM1 1640 (media culture) (Logan, UT, USA) for FCI, while another portion was collected into a container containing formalin for histopathology examination (HPE). In the flow cytometry laboratory, the lymph node tissues were processed and cell suspensions were prepared. The cell suspensions were then analyzed using FACSCalibur (Becton Dickinson, San Diego, CA, USA), and the data were analyzed using Paint-a-Gate software (Becton Dickinson, San Diego, CA, USA). The findings of flow cytometry analysis were compared with tissue histological diagnosis.

Within the 12 months of study, only 11 lymph node biopsies were available for analysis. There were 9 males and 2 females involved in this study. Their age ranged from 18-74 years old. Most of the cases had presented with multiple lymph node enlargement (7/11; 64%). Out of 11 samples, 9 (82%) were positive to CD45, which was consistent with hemopoietic cells. Another 2 samples (18%) were negative towards CD45 as well as other monoclonal antibodies. In view of their clinical findings, diagnoses of non-hemopoietic neoplasms were made. Five out of 9 cases with CD45 positivity showed strong expression towards CD19 and CD20, screening panel for B lineage. Two cases showed an immunophenotype that was typical of small lymphocytic lymphoma/chronic lymphocytic leukemia (case one and 11 with CD23+, CD5+, CD22+, surface Ig weak with negative FMC7) (**Table 1**). In another 3 cases, the diagnoses of B-NHL were made as flow cytometric was able to detect the B neoplastic cells. One case showed positivity towards kappa with absent lambda expression (kappa light chain restriction) (case 5), one showed abnormal kappa; lambda ratio (case 6) and another one showed immunophenotypic aberration (case 10) where no expression of surface light chain immunoglobulin was present (**Table 1**). Two cases (18%) were positive towards T-cell lineage, in which the dominant cell population (>90%) was positive towards cytoplasmic CD3 in the screening panel. One case was diagnosed as T-lymphoblastic leukemia/lymphoma (case 3) as the population was positive towards CD34, intraTdT, in addition to the T-cell markers. Another case (case 8) was diagnosed as T-lymphoproliferative disease as the predominant

Table 1 - Clinicopathologic data and flow cytometric immunophenotyping findings of lymph node specimens processed in the Hematology and Histopathology Laboratories, Diagnostic Laboratory Services Department, UKMMC, Kuala Lumpur, Malaysia.

Case no.	Gender/ Age	Site of LN biopsies	FCM Phenotype	FCM Kappa/ lambda ratio	FCM diagnosis	Immunohistochemistry	Histology diagnosis
1	M/57	Cervical	CD19+,CD20+,CD23+,CD5+, surIgG+	kappa*	SLL	CD20+, CD79a+, CD3+	SLL
2	M/52	Cervical	CD45-,cCD3-, CD19-,CD20-, CD56-	ND	Non-hemopoietic neoplasm	CK7+,TTF-1+,CD20-	Metastatic carcinoma most likely from the lung
3	M/19	Inguinal	CD3+,CD5+,CD7+,CD34+,TDT+	NA	T-cell lymphoblastic leukemia/ Lymphoma	CD3+,CD20-,CD79a-	T-cell lymphoblastic leukemia/lymphoma
4	M/65	Cervical	CD45-,cCD3-,CD19-,CD20, CD56-	-	Non-hemopoietic neoplasm	CK7+,LCA-	Metastatic undifferentiated arcinoma
5	M/71	Inguinal	CD19+,CD20+,CD23+, surIgM+	3.3	B-NHL	bcl-2: inconclusive CD20: inconclusive	Follicular lymphoma
6	F/42	Cervical	CD19+,CD20+,CD23+,surIgM+	kappa*	B-NHL	Bcl-2+ CD20+	Follicular lymphoma
7	F/19	Cervical	CD19+,CD20+,cCD3+,CD15-,CD 30-HLA-DR+	1.12	Inconclusive	CD30+CD15-	Hodgkin's lymphoma, mixed cellularity
8	M/18	Inguinal	cCD3+,CD5+,CD2+,CD7+, CD8+CD4+,CD25+	NA	T-cell lymphoproli-ferative disease.	CD3+,CD20-,CD79a- CD30+	Anaplastic T-cell lymphoma
9	M/25	Axillary	CD19+,CD20+cCD3+,CD15, CD30-HLA-DR+	1.3	Inconclusive	CD15+,CD30+, CD3-CD20-	Hodgkin's lymphoma, mixed cellularity
10	M/74	Inguinal	CD19+,CD20+,cCD3-,CD22+	-	Suspected B-NHL	CD20,CD79a+LCA:+,CD3-	DLBCL
11	M/60	Cervical	CD19+,CD20+,CD5+,CD23+, FMC7-,surIgM+	kappa*	SLL/CLL	CD20+,CD3-ki67:95%	DLBCL (transformation from SLL/CLL)

*kappa light chain restriction, ND - not detected, NA - not available, M - male, F - female, NHL - non Hodgkin lymphoma, FCM - flow cytometric immunophenotyping, DLBCL - diffuse large B-cell lymphoma, SLL - small lymphocytic lymphoma, LN - lymph node, CLL - chronic lymphocytic leukemia

population was T lymphocytes with overexpression of surface CD3 (**Table 1**). Two known cases of Hodgkin's lymphoma were inconclusive by flow cytometry. There was an absence of a population that expressed CD45, moderate SSC (intermediate granularity), CD15 and CD30 were negative. Analysis from both cases showed abundance of T- and B-lymphocytes (**Table 1**).

For several years, FCI has maintained its position as an indispensable diagnostic tool in hematologic neoplasms.[5] The FCI is particularly useful in heterogenous cell populations where the subpopulation of interest can be selected electronically and analyzed independently. For B-cell neoplasms, a pure population of light chain restricted B-cells was easily recognized using flow cytometry (cases one, 6, and 11) (**Table 1**), and was usually reflected by an abnormal kappa/lambda ratio. False-negative flow cytometric evaluation could occur in some tumors which contain few neoplastic cells (for example the T-cell/histiocyte rich variant of diffuse large B-cell lymphoma), or many admixed reactive B-cells (for example marginal zone lymphoma)[5] and also in cases of Hodgkin lymphoma. Hence, it is important to acquire enough events to detect the small population of an abnormal cells (30,000 - 50,000). In our study, the acquisition of events was set at 10,000 although some of the cases had abundance of cells. In the cases of Hodgkin lymphoma, flow cytometric evaluation always yield a negative result as the neoplastic cells were very few in the background of reactive cells. Therefore, identification of the Reed-Sternberg was undoubtedly difficult, although some reports recommended the use of CD15 and CD30 to aid in the detection of the neoplastic cells.[5] However, in our cases, both showed negative results. There were 2 cases of non-hemopoietic neoplasms (case 2 and 4) (**Table 1**). Flow cytometry analysis from lymph nodes biopsy showed that the malignant cells were negative to all panels of monoclonal antibodies of hemopoietic markers (CD45-, cCD3-, CD19-, CD20-, CD56-). Diagnoses of metastatic cancerous lung lesion and

undifferentiated metastatic carcinoma of the lymph nodes were confirmed by histology diagnosis.

Both HPE and immunohistochemistry of lymph nodes tissue remain a gold standard for diagnosis of lymphoma and metastatic carcinoma. However, results generated by FCI are faster as the whole procedure takes only a few hours and analysis can be carried out almost immediately. The paraffin embedded immunohistochemistry procedure usually takes several days and results generation usually takes a few days after the procedures. This study exemplified some of the unique abilities of FCI in the recognition and identification of non-Hodgkin's lymphoma in tissue-based specimens. Time factor is also another advantage of using flow cytometric analysis, where this technique generates faster results.

In conclusion, our study showed that flow cytometric analysis on lymph node tissue was technically simpler and generated faster results. Thus, this method provides another option for analysis when problems arise in obtaining tissue samples, or urgent diagnosis is required. This technique is able to recognize cases of NHL, however cases of Hodgkin lymphoma remained difficult to diagnose by flow cytometry.

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From the Department of Pathology (Raja-Sabudin, Yusof, Alauddin, Aziz, Kulaveerasingam, Zin, Ali, Othman, Hussin), Department of Surgery (Muhammad), Department of Anatomy (Das), Faculty of Medicine, University Kebangsaan Malaysia Medical Centre, Department of Pathology (Hamid), Hospital Sultan Ismail, Johor, Department of Medical Sciences II (Othman), Faculty of Medicine and Health Sciences, University Islam Sains Malaysia, Kuala Lumpur, Malaysia. Address correspondence and reprints request to: Dr. Raja-Zahratul A. Raja-Sabudin, Department of Pathology, University Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak 56000, Kuala Lumpur, Malaysia. Tel. +603 (9) 1455555 Ext. 5780/5356. Fax. +603 (9) 1737340. E-mail: zahratul@ppukm.ukm.edu.my

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