Flow cytometry of lymph node aspirate can effectively differentiate the reactive lymphadenitis from the nodal non-Hodgkin lymphoma

Bishnu Pada Dey, Saumitra Chakravarty and Mohammed Kamal

Article Info

Department of Pathology, Faculty of Basic Science and Paraclinical Science, Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka, Bangladesh

For Correspondence:
Bishnu Pada Dey
drbishnuk51@gmail.com

Received: 2 September 2017
Accepted: 16 November 2017
Available Online: 25 November 2017

ISSN: 2224-7750 (Online)
2074-2908 (Print)

DOI: 10.3329/bsmmuj.v10i4.34560

Cite this article:
Dey BP, Chakravarty S, Kamal M. Flow cytometry of lymph node aspirate can effectively differentiate reactive lymphadenitis from nodal non hodgkin lymphoma. Bangabandhu Sheikh Mujib Med Univ J. 2017; 10: 219-26.

Available at:
www.banglajol.info

A Journal of Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

Abstract

The purpose of the present study was to compare the routine cytology, histology, immunohistochemistry and flow cytometry in the diagnosis of nodal lymphoma cases. Thirty five cases of clinically suspected lymphoproliferative disorder were included in this study. After preparation of smears from the fine needle aspirates on the glass slides for cytology, the residual material was processed for flow cytometric immunophenotyping accordingly. Subsequently, histopathology and immunohistochemistry findings of selected cases of lymph node biopsy were correlated to confirm and compare the diagnosis. Flow cytometric immunophenotypes of most of the cases corresponded to the histological and immunohistochemical diagnoses, five cases showed marked shift in diagnosis (e.g. aggressive NK cell leukemia) which were later validated by clinical outcomes. Flow cytometry immunophenotyping had shown high sensitivity (96.4%) and specificity (100%) if we consider both histopathology and immunohistochemistry combined as gold standard, while in comparison to immunohistochemistry, flow cytometry immunophenotyping had shown 100% sensitivity and specificity. However, despite the improved diagnostic capacity provided by flow cytometry, morphologic analysis obtained from histopathology remains the cornerstone in the diagnosis of lymphoma.

Introduction

Lymphoma is a common malignancy worldwide affecting both children and adults. In Bangladesh, non-Hodgkin lymphoma (NHL) is relatively common and contributing to 7% of total cancer reported[1] as compared to 4% in USA.[2] Nodal lymphoma is rather prevalent in Bangladesh; for instance, lymphoma constitutes about 6% of all the cases of cervical lymphadenopathy persisting for more than three weeks.[3] Although fine needle aspiration cytology can diagnose almost all the cases of reactive lymphadenitis, only up to about 86% cases of NHL can be correctly identified by cytology alone.[4]

Fine needle aspiration cytology is accepted in recurrence lymphoma; primary diagnosis and classification of NHLs are usually made by histological examination and till date, considered to be the gold standard. Worldwide, many centers have reported fine needle aspiration cytology show relatively high sensitivity and specificity in the diagnosis of lymphomas when used in conjunction with other ancillary techniques in last 15 years.[5,6] Despite this, non-Hodgkin lymphoma diagnosis of on fine needle aspiration material has remained controversial.[7-9] Even though architectural and morphologic feature continues to be important parameters in World Health Organization's classifycation of hematopoietic and lymphoid tumors, lymphoma diagnosis is now routinely based on combined information from clinical manifestations, histomorphologic features, immunophenotype and genotype.[10, 11, 12] Flow cytometric immunophenotyping offers many advantages in the analysis of fine needle aspiration materials for lymphoma diagnosis. Moreover, it requires small number of cells and is also suitable for cells already in suspension. It is very sensitive immunophenotyping technique, and can detect aberrant cells even 1/10,000 cells.[13] Thus, it almost replaced the classic immunocytochemistry.[14] As in immunocytochemistry only one antigen can be assessed per cell at a time, it is rather time consuming. Large number of specific monoclonal anti-bodies combining four or more fluorochromes can precisely define the cell profile and neoplastic nature of lymphoid cells make FCI is a more sensitive, specific and swift diagnostic tool than is immunocytochemistry.[13, 15-17]

Indeed FCI evaluates several antigens on one cell (including cytoplasmic antigens), and can

detect small aberrant cells populations in a reactive background gives quantitative results, it can also assess co-expression of antigens on subsets of cells and can measure relative density of surface antigens. It can also identify very few abnormal cells that may not be apparent on morphology. These features significantly improve the diagnostic sensitivity, specificity of lymphoma diagnosis.[18, 19] Present study aims to address the issue by comparing diagnosis of nodal lymphoma cases across different diagnostic platforms including routine cytology, histology, immunohistochemistry and flow cytometry.

Materials and Methods

This study was carried out from November 2015 to December 2016. FCI were carried out with Department of Microbiology and Immunology. For this purpose, a total of 35 consecutive cases of clinically suspected lymphoproliferative disorder were included. After preparation of smears from the fine needle aspirates on the glass slides, the residual material was processed for flow cytometric immunophenotyping according to BD FACS Verse™ manual (2013). Subsequently, histopathology and immunohistochemistry reports of selected cases of lymph node biopsy were collected to confirm and compare the diagnosis. Routine HE stain was used in histology. FNAC slides were also stained with Hematoxylin and Eosin (HE) stain along with Papanicolaou stain. Markers used for immunohistochemistry and flow cytometry are listed in the Table I. The diagnostic criteria used for flow cytometric immunophenotyping of lymphoma are summarized in the Table II and III.[20-22] All the results were recorded according to revised WHO classification of tumors of hematopoietic and lymphoid tissues (2016) were used throughout the study.[12, 22]

Results

Among the 35 cases, 28 were males and 7 were females. The average ages of male and female patients were 47.7 ± 17.7 and 31.9 ± 13.3 years, respectively (p = 0.013), while the average age regardless of sex was 44.5 ± 17.9 years. Cervical lymph node was the most frequent (66%) site of sample collection. FNAC diagnosis revealed lymphoproliferative disorder as the most frequent (46%) entity among the samples under study.

Forty three percent cases were diagnosed as non-Hodgkin lymphoma without further lineage-specific subclassification, 17% cases as lymphoma of B cell lineage, 6% cases as lymphoma of B cell lineage, 6% cases as suspected lymphoma and 14% cases as other than lymphoma by histopathology alone. Histopathology reports or required tissue sample of 14% cases were not available. When the histological data were combined with immunohistochemistry, it revealed 34% and 11% cases of non Hodgkin lymphoma with B and T cell lineages, respectively, while 6% cases were still indeterminate regarding lineage. Besides, 9% cases were diagnosed as something other than lymphoma but combined histological and immunohistochemical inference were not available in 40% cases. Finally, flow cytometry subclassified 51% and 32% cases of non-Hodgkin lymphoma into B and T cell lineage, respectively, and left none of the cases non-specific of lineage. 17% cases, however, were found to be inconsistent with lymphoma.

It is to be noted that, among the 35 cases 91% cases were primary and 9% cases were recurrent cases of lymphoma. Among the recurrent cases two were B-cell type non-Hodgkin lymphoma and one was Hodgkin lymphoma. All of those three recurrent cases were diagnosed definitely as positive for lymphoma by flow cytometry.

The changes of diagnoses across histopathology only to combine histological and immunohistochemical inference to ultimately flow cytometry found in this study are depicted in the Table IV. The diagnoses of some of the cases, i.e. follicular lymphoma and small lymphocytic lymphoma, remained unchanged across the aforementioned three platforms while radical changes in diagnosis was observed in a few cases, e.g. a case of non-Hodgkin lymphoma diagnosed initially by histopathology later diagnosed as mixed cellularity classical Hodgkin lymphoma by immunohisto-

Table I

Markers used in immunohistochemistry (IHC) and flow cytometric immunophenotyping (FCI)

B cell marker	IHC	FCI
	CD20, CD79α, PAX5, CD21, CD23, CD43, CD10, BCL2, BCL6, Kappa and Lambda light chains, TdT	CD19, CD5, CD20, CD22, CD23, CD10, CD79β, FMC7, Kappa and Lambda light chains, cBCL2
T cell marker	CD2, CD3, CD4, CD5, CD7, CD8, TdT	CD2, CD3, CD4, CD5, CD7, CD8, ALK
NK cell marker	CD56	CD16, CD56, CD57
Hodgkin lymphoma marker	CD15, CD30	CD15, CD30, CD40, CD95, CD71
Proliferative index marker	Ki67	Not applicable

Table II

Immunophenotypic criteria for classification of B-cell lymphoma

Diagnosis	CD19	CD5	CD23	CD20	CD10	CD15	CD30	K/L*
Follicular lymphoma	+	-	-	+	+	NA	NA	Clonal
Chronic lymphocytic leukemia/Small lymphocytic lymphoma	+	+	+	+(weak)	-	NA	NA	Clonal
Immunocytoma/lymphoplasmocytic lymphoma	+	-	-	+	-	NA	NA	Clonal
Extranodal marginal zone B-cell lymphoma of mucosaassociated lymphoid tissue type/ nodal marginal zone lymphoma	+	-	-	+	-	NA	NA	Clonal
Mantle cell lymphoma	+	+	-	+	-	NA	NA	Clonal
High-grade B-cell non-Hodgkin lymphoma	+	-	-/+	+/-	+/-	-	-/+	Clonal
Classical Hodgkin lymphoma	+	-	NA	-/+	NA	+ (RS)[¢]	+(RS)[¢]	NA
Nodular lymphocyte predominant Hodgkin lymphoma	+	-	NA	+ (LH)[ω]	NA	-	-	NA
Reactive hyperplasia	+	-	-/+	+/-	-/+	NA	NA	PC

* K/L: kappa/ lambda light-chain ratio; [ω]LH: lymphocytic/histiocytic cell; RS: [¢]Reed-Strenberg cell

Table III

Immunophenotypic criteria for classification of T-cell lymphoma

Diagnosis	CD2	CD3	CD5	CD7	CD10	ALK	BCL2	CD30	CD20	CD4/CD8
Peripheral T cell lymphoma	+	+	-	-	-	-	+/-	-/+	NA	>1
Angioimmunoblastic lymphoma	+	+	+		+/-	-	+/-		+	>1
Adult T cell lymphoma	+	+	+	-	-	-	NA	-/+	NA	>1
Anaplastic large cell lymphoma	-/+	-/+	NA	-/+	-	+	NA	+	-	NA
B cell rich T cell lymphoma	-	-	NA	NA	NA	-	NA	-	+	NA
Precursor T lymphoblastic lymphoma	+/-	+/-	+/-	+/-	+	-	NA	NA	NA	NA
T zone hyperplasia	+/-	+	+	+/-	-	-	-	+ (variable)	+	1

chemistry; but reverted to angioimmunoblastic T cell lymphoma by flow cytometry (Figure 1). The treatment response validated the diagnosis. Shifts of diagnoses towards gradual subclassification are also seen, i.e. two cases diagnosed as non-Hodgkin lymphoma by histopathology were subcategorized as non-Hodgkin lymphoma of B cell lineage using immunohistochemistry in combinations which were then further specified as diffuse large B cell lymphoma by flow cytometry.

Discussion

The histopathology diagnosis had shown that sensitivity, specificity, positive predictive value and negative predictive value were 94%, 50%, 94.1% and 50% respectively; While the flow cytometry immunophenotyping diagnosis had very high sensitivity 96.4% and specificity, accuracy, strength, positive predictive value, negative predictive value were 100%, 96.9%, 98.2%, 100% and 80% respectively if we considered both histopathology and immunohistochemistry combined as a gold standard.

Moreover, in comparison to immunohistochemistry, flow cytometry immunophenotyping had shown 100% sensitivity, specificity, accuracy, strength, positive predictive value and negative predictive value.

Multiparameter flow cytometric immunophenotyping has significantly enhanced the diagnostic role of FNA, particularly in the case of hematolymphoid malignancies, playing a fundamental role in the differential diagnosis between reactive processes and lymphomas. This study showed that specific and sensitive identification of neoplastic cells, their accurate enumeration, and

Table IV

Evolution of diagnosis across histopathology, immunohistochemistry and flow cytometry

Routine histology only	Histopathology with Immunohisto-chemistry	Flow cytometry	Number of cases
Non-Hodgkin lymphoma	Non Hodgkin lymphoma	Diffuse large B-cell lymphoma	1
		Small lymphocytic lymphoma	1
Non-Hodgkin lymphoma	Non Hodgkin lymphoma of B cell line-age	Diffuse large B-cell lymphoma	2
Non-Hodgkin lymphoma	Diffuse large B-cell lymphoma	Diffuse large B-cell lymphoma	3
Non-Hodgkin lymphoma	Mantle cell lymphoma	Mantle cell lymphoma	1
Non-Hodgkin lymphoma	Not d	Small lymphocytic lymphoma	1
	Mixed cellularity classical Hodgkin lymphoma	Angioimmunoblastic T-cell lymphoma	1
	Peripheral T-cell lymphoma, NOS[a]	Adult T-cell lymphoma	1
	Myeloid sarcoma	Aggressive NK cell leukemia	1
	Not available	Aggressive NK cell leukemia	1
		Diffuse large B-cell lymphoma	1
		Not consistent with lymphoma	1
Angioimmunoblastic T-cell lym-phoma	Non Hodgkin lymphoma of T cell line-age	Anaplastic large cell lymphoma, ALK positive	1
	Not available	Angioimmunoblastic T-cell lymphoma	1
Follicular lymphoma	Follicular lymphoma	Follicular lymphoma	1
Small lymphocytic lymphoma	Small lymphocytic lymphoma	Small lymphocytic lymphoma	1
Mixed cellularity classical Hodg-kin lymphoma	Mixed cellularity classical Hodgkin lymphoma	Peripheral T-cell lymphoma, NOS	1
	Peripheral T-cell lymphoma, NOS	Peripheral T-cell lymphoma, NOS	1
	Not available	Angioimmunoblastic T-cell lymphoma	1
	Not available	Small lymphocytic lymphoma	1
Lymphoproliferative disorder	Not available	Not consistent with lymphoma	1
Atypical lymphoid hyperplasia	Not available	Diffuse large B-cell lymphoma	1
Chronic nonspecific lymphade-nitis	Peripheral T-cell lymphoma, NOS	Peripheral T-cell lymphoma, NOS	1
	Not available	Precursor T-lymphoblastic lymphoma	1
		Not consistent with lymphoma	1
Granulomatous inflammation	Chronic nonspecific lymphadenitis	Not consistent with lymphoma	1
Sarcoma	Rhabdomyosarcoma	Not consistent with lymphoma	1
Not available	Non Hodgkin lymphoma of B cell line-age	Diffuse large B-cell lymphoma	1
	Not available	Diffuse large B-cell lymphoma	2
		Classical Hodgkin lymphoma	1
		Not consistent with lymphoma	1
Total			**35**

[a]not otherwise specified

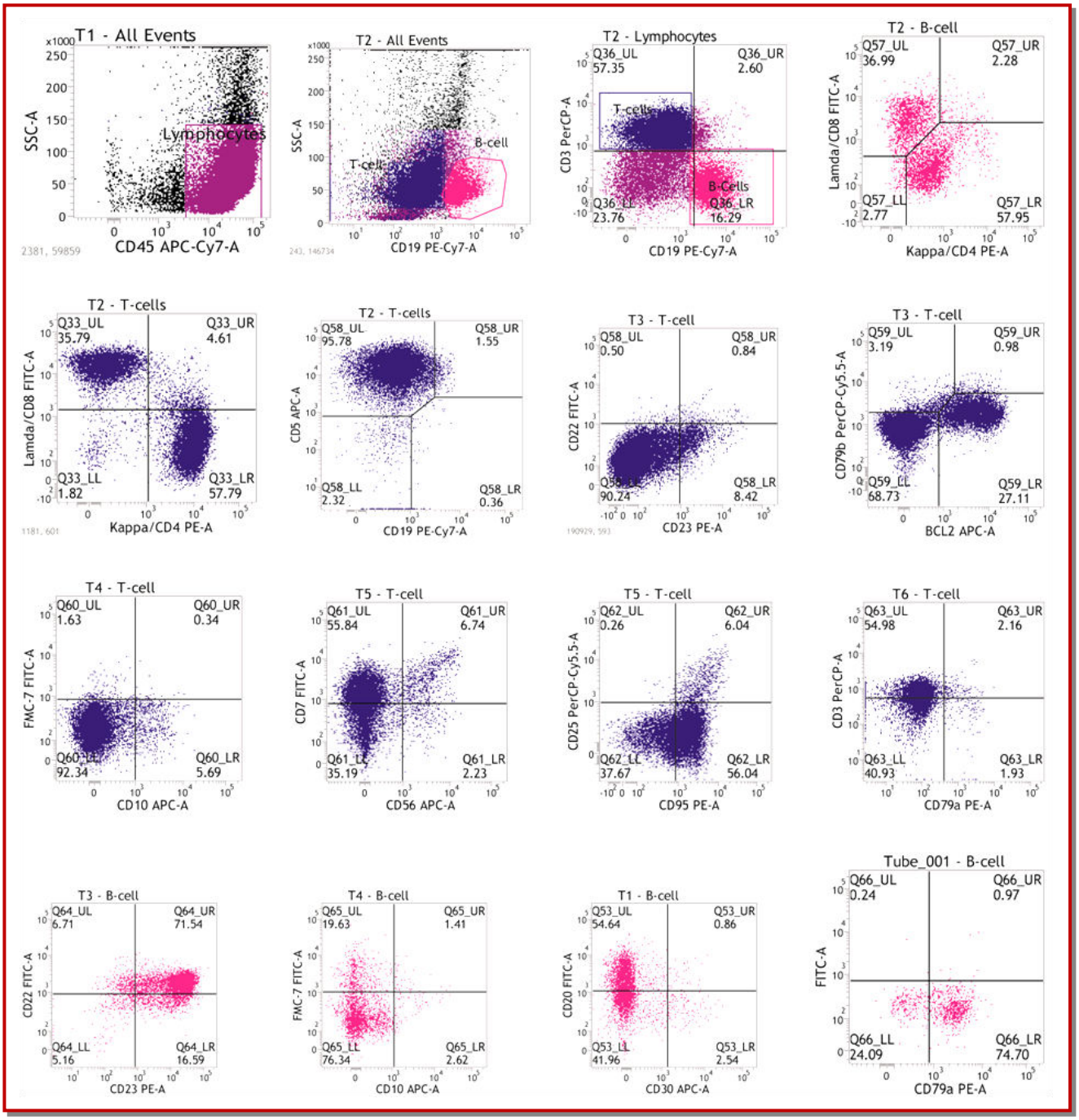

Figure 1: FCI scatter plots of the lymph node aspirate from a representative case angioimmunoblastic T-cell lymphoma

subclassification can be effectively provided by modern flow cytometry techniques. The ability to gain more information from a smaller amount of tissue, with high-level multicolor FC, should reduce the number of inadequate results on specimens in which the number of cells is a limiting factor, such as fine-needle aspirates.

In the case of deep abdominal or intrathoracic lesions, where a surgical biopsy is not possible, the combined use of FNA cytology and FC proved to be a very useful diagnostic tool.

On the other hand, to gain rapid and reliable diagnosis and treatment decisions by the FCI may represent a true cost-effective advantage in developing countries like Bangladesh, where economic resources are limited to include lymph-node biopsy in diagnostic procedures.

Diagnoses variations across different methods are usual due to the varying amounts of informa-

tion or levels of precision associated with the method, especially of oncology diagnostics. Sometimes the change may refer to a subclassification of a broader category while occasionally there are recategorizations.[23] This study discuss mainly towards possible explanations and potential implications of the observed shifts of diagnostic categorization across different platforms.

Due to pleomorphic appearance of T lymphocytes at the background, it is not uncommon to misdiagnose T cell lymphomas as Hodgkin lymphoma by histopathology. If the follicular dendritic cell proliferation and prominent high endothelial venules are not typical enough in conjunction with Epstein-Barr virus (EBV) positive immunoblasts masquerading as Reed-Sternberg like cells, angioimmunoblastic T cell lymphoma might mimic mixed cellularity classical Hodgkin lymphoma, as evidenced by two cases of the present study.[24, 25] Anaplastic large cell lymphoma is regarded as another differential diagnosis of angioimmunoblastic T cell lymphoma, exemplified by a case in the present study due to the fact that both might manifest endothelial proliferation.[26, 27] Peripheral T cell lymphoma is another mimicker of mixed cellularity classical Hodgkin lymphoma because the former often shows Reed-Sternberg like cells, as seen in at least two cases of the present study.[28] It is to note that, the cases which were initially diagnosed as Hodgkin lymphoma by histopatholgy and/or immunohistochemistry but later diagnosed as peripheral T cell lymphoma by FCI, were unresponsive to the conventional ABVD therapy but responded promptly upon the administration of appropriate therapy for peripheral T cell lymphoma in the light of FCI diagnosis.

Mantle cell lymphoma can be difficult to differentiate from small lymphocytic lymphoma because of the vague nodularity and neoplastic small cells shared by both as well as their near-similar immunohistochemical profiles where both CD23 and CD10 might show positivity necessitating flow cytometric measurement to determine the cut-off of their expression levels in order to tell the difference.[29] Due to seemingly inconspicuous morphology albeit poor prognosis, it is often impossible to diagnose NK cell lymphoma/leukemia with histopathology alone which was shown in two cases of the present study those required the help of flow cytometry.[30]

Lymphomas may also mimic benign conditions like chronic nonspecific lymphadenitis which is not too uncommon in medical literature. Two of the cases in the present study were diagnosed as such at first by later revealed by flow cytometry as peripheral T cell lymphoma and precursor T lymphoblastic lymphoma, respectively. The initial under diagnosis was probably due to the difficulty to distinguish between the morphologies of lymphoma cells and that of lymphocytes with reactive atypia as well as between the effacement of nodal architecture and paracortical hyperplasia of the lymph node which often misleads the pathologists.[31, 32]

The validity of the diagnoses provided by flow cytometry, especially in cases where Hodgkin lymphomas were reclassified as non-Hodgkin lymphoma and cases of chronic nonspecific lymphadenitis being recategorized as T cell lymphoma, were assured by the improved treatment response after the therapies were reshaped and redesigned in the light of the new diagnoses. Unfortunately, one of the cases which were recategorized by flow cytometry as aggressive NK cell lymphoma died shortly after, probably highlighting the higher diagnostic utility of the method. These represent the importance of incorporating flow cytometry as an integral part of frontline lymphoma diagnostics.

The discussion so far emphasizes the need to consider appropriate differential diagnosis of lymphoma and highlights the necessity of advanced immunophenotyping methods like flow cytometry. While conventional morphological methods like histopathology and immunohistochemistry remain at the core of the lymphoma diagnostic array, flow cytometry as an adjunct offers the required accuracy and precision to help achieve a higher degree of favorable outcome.

Conclusion

Flow cytometry immunophenotyping (FCI) is a sensitive and specific method in diagnosis and classification of NHL as well as in detection of monoclonality. FCI also can effectively differentiate reactive lymphadenitis from nodal NHL. The result of FNAC in combination with FCI is effective in the diagnosis of NHL with complete subclassification. We can avoid expensive surgical biopsies in majority of cases.

Ethical Issue

The research protocol was approved by institutional review board of BSMMU (No. BSMMU/2016/3458;

Date: 23.3.2016). Written and informed consent was taken from each of the study subjects or their lawful guardians (wherever appropriate) for publishing the study.

Conflict of interest

There is no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

Acknowledgement

We owe our heartfelt gratitude and indebtedness to Dr. Shirin Tarafder (Associate Professor), Dr. Khondoker Hafiza Khanom (Resident) and Md Yunus Ali (Scientific officer), of the Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University.

References

1. Mahmud F. Establishment of pathology based tumor registry at BSMMU [thesis]. Dhaka, Bangladesh: Department of Pathology, Bangabandhu Sheikh Mujib Medical University, 2014.

2. SEER Cancer Statistical Review, 1973-1999. 2002. Available at: https://seer.cancer.gov/archive/csr/1973_1999/. Accessed July 31, 2017.

3. Kafi AH, Arif HB, Ruhul AS. Role of fine needle aspiration cytology in the diagnosis of cervical lymphadenopathy. Bangladesh J Med Sci. 2012; 11: 25-27.

4. Islam MR, Hoque MA, Ekram AS. A study of peripheral lymphadenopathy with correlation between fine needle aspiration cytology (FNAC) and biopsy in a tertiary care hospital. Bangladesh J Med. 2015; 26: 49-54.

5. Mathiot C, Decaudin D, Klijanienko J, Couturier J, Salomon A, Dumont J, Vielh P. Fine-needle aspiration cytology combined with flow cytometry immunophenotyping is a rapid and accurate approach for the evaluation of suspicious superficial lymphoid lesions. Diagn Cytopathol. 2006; 34: 472-78.

6. Dunphy C, Ramos R. Combining fine-needle aspiration and flow cytometric immuno-phenotyping in evaluation of nodal and extranodal sites for possible lymphoma: A retrospective review. Diagn Cytopathol. 1997; 16: 200-06.

7. Hehn S, Grogan T, Miller T. Utility of fine-needle aspiration as a diagnostic technique in lymphoma. J Clin Oncol. 2004; 22: 3046-52.

8. Landgren O, Porwit MacDonald A, Tani E, Czader M, Grimfors G, Skoog L, Ost A, Wedelin C, Axdorph U, Svedmyr E, Bjorkholm M. A prospective comparison of fine-needle aspiration cytology and histopathology in the diagnosis and classification of lymphomas. Hematol J. 2004; 5: 69-76.

9. Wakely P. Fine-needle aspiration cytopathology in diagnosis and classification of malignant lymphoma: Accurate and reliable? Diagn Cytopathol. 2000; 22: 120-25.

10. Chan JK, Banks PM, Cleary ML, Delsol G, De Wolf-Peeters C, Falini B, Gatter KC, Grogan TM, Harris NL, Isaacson PG, Jaffe ES. A revised European-American classification of lymphoid neoplasms proposed by the International Lymphoma Study Group: A summary version. Am J Clin Pathol. 1995; 103: 543-60.

11. Robins D, Katz R, Swan F, Atkinson E, Ordonez N, Huh Y. Immunotyping of lymphoma by fine-needle aspiration: A comparative study of cytospin preparations and flow cytometry. Am J Clin Pathol. 1994; 101: 569-76.

12. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J (eds). WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon, WHO/IARC, 2015, p 2.

13. Jorgensen J. State of the Art Symposium: Flow cytometry in the diagnosis of lymphoproliferative disorders by fine-needle aspiration. Cancer Cytopathol. 2005; 105: 443-51.

14. Mayall F, Dray M, Stanley D, Harrison B, Allen R. Immunoflow cytometry and cell block immunohistochemistry in the FNA diagnosis of lymphoma: A review of 73 consecutive cases. J Clin Pathol. 2000; 53: 451-57.

15. Caraway N. Strategies to diagnose lymphoproliferative disorders by fine-needle aspiration by using ancillary studies. Cancer Cytopathol. 2005; 105: 432-42.

16. Simsir A, Fetsch P, Stetler-Stevenson M, Abati A. Immunophenotypic analysis of non-Hodgkin's lymphomas in cytologic specimens: A correlative study of immunocytochemical and flow cytometric techniques. Diagn Cytopathol. 1999; 20: 278-84.

17. Zardawi I, Jain S, Bennett G. Flow-cytometric algorithm on fine-needle aspirates for the clinical work-up of patients with lymphadenopathy. Diagn Cytopathol. 1998; 19: 274-78.

18. Orell S, Sterrett G, Walters MI, Whitaker D. Manual and atlas of fine needle aspiration cytology. 2nd ed. Edinburgh, Churchill Livingstone, 1992.

19. Stacchini A, Demurtas A, Godio L, Martini G, Antinoro V, Palestro G. Flow cytometry in the bone marrow staging of mature B-cell neoplasms. Cytometry B Clin Cytom. 2003; 54: 10-18.

20. Laane E, Tani E, Björklund E, Elmberger G, Everaus H, Skoog L, Porwit-MacDonald A. Flow cytometric immunophenotyping including Bcl-2 detec-

tion on fine needle aspirates in the diagnosis of reactive lymphadenopathy and non-Hodgkin's lymphoma. Cytometry B Clin Cytom. 2005; 64: 34-42.

21. Pileri SA, Ascani S, Leoncini L, Sabattini E, Zinzani PL, Piccaluga PP, Pileri A, Giunti M, Falini B, Bolis GB, Stein H. Hodgkin lymphoma: The pathologists viewpoint. J Clin Pathol. 2002; 55: 162-76.

22. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, Advani R, Ghielmini M, Salles GA, Zelenetz AD, Jaffe ES. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood 2016; 127: 2375-90.

23. Pileri SA, Ascani S, Leoncini L, Sabattini E, Zinzani PL, Piccaluga PP, Pileri A, Giunti M, Falini B, Bolis GB, Stein H. Hodgkin lymphoma: The pathologists viewpoint. J Clin Pathol. 2002; 55: 162-76.

24. Sachsida-Colombo E, Mariano LC, Bastos FQ, Rassi AB, Lage LA, Barreto A, Siqueira S, Pereira J. A difficult case of angioimmunoblastic T-cell lymphoma to diagnose. Revista Brasileira de Hematologia e Hemoterapia. 2016; 38:82-5.

25. Laforga J, Gasent J, Vaquero M. Potential misdiagnosis of angioimmunoblastic T-cell lymphoma with Hodgkin's lymphoma: A case report. Acta Cytol. 2010; 54: 840-44.

26. Feldman AL, Law ME, Inwards DJ, Dogan A, McClure RF, Macon WR. PAX5-positive T-cell anaplastic large cell lymphomas associated with extra copies of the PAX5 gene locus. Mod Pathol. 2010; 23: 593–602.

27. Kesler M, Paranjape G, Asplund S, McKenna R, Jamal S, Kroft S. Anaplastic large cell lymphoma: A flow cytometric analysis of 29 cases. Am J Clin Pathol. 2007; 128: 314-22.

28. Asano N, Kinoshita T, Tamaru JI, Ohshima K, Yoshino T, Niitsu N, Tsukamoto N, Hirabayashi K, Izutsu K, Taniwaki M, Morishima Y. Cytotoxic molecule-positive classical Hodgkin's lymphoma: a clinicopathological comparison with cytotoxic molecule-positive peripheral T-cell lymphoma of not otherwise specified type. Haematologica 2011; 96: 1636-43.

29. Gong J, Lagoo A, Peters D, Horvatinovich J, Benz P, Buckley P. Value of CD23 determination by flow cytometry in differentiating mantle cell lymphoma from chronic lymphocytic leukemia/small lymphocytic lymphoma. Am J Clin Pathol. 2001; 116: 893-97.

30. Asile D, Vladareanu AM, Bumbea H. Peripheral T and NK cell non-Hodgkin lymphoma a challenge for diagnosis. Maedica (Buchar). 2014; 9: 104-08.

31. Jamal S, Picker LJ, Aquino DB, McKenna RW, Dawson DB, Kroft SH. Immunophenotypic analysis of peripheral T-cell neoplasms: A multiparameter flow cytometric approach. Am J Clin Pathol. 2001; 116: 512-26.

32. Gorczyca W, Weisberger J, Liu Z, Tsang P, Hossein M, Wu CD, Dong H, Wong JY, Tugulea S, Dee S, Melamed MR. An approach to diagnosis of T-cell lymphoproliferative disorders by flow cytometry. Cytometry Part A. 2002; 50: 177-90.