Detection of Lymphoid Neoplasms in Bone Marrow by Flow Cytometric Analysis and Comparison to Bone Marrow Aspiration and Biopsy

Orathai Promsuwicha MSc*,
Wayuree Songmuang BSc*, Chirayu U Auewarakul MD, PhD*

** Division of Hematology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*

Background: *Lymphoid neoplasms are a heterogeneous group of hematologic malignancies. Bone marrow (BM) can be involved in a certain proportion of lymphoid neoplasms, necessitating accurate and rapid diagnosis of such involvement for early therapeutic decision.*

Objective: *To evaluate the diagnostic utility of flow cytometry (FC) for assessment of BM involvement by lymphoid neoplasms. BM biopsy (BMBx) was used as the gold standard and BM aspiration (BMA) was used as a comparison.*

Material and Method: *Two hundred and eighty-three samples with a clinical suspicion for lymphoid neoplasms were received in FC laboratory and analysed using various lymphoid markers. The FC results were then compared to BMA and BMBx.*

Results: *Of 283 cases, 94 had lymphoid neoplasms by BMBx (33%). Among the positive BMBx cases, concordant agreement of all three investigations was found in 45 cases (48%). FC was positive in 52/94 cases (55%) while BMA was positive in 62/94 cases (66%). Among the negative BMBx cases, FC was positive in 8/189 cases (4%) and BMA was positive in 56/189 cases (30%). FC and BMA were both negative in 25/94 cases (27%). The specificity of FC and BMA was 96% and 60% while the sensitivity was 55% and 65%, respectively. Subtype agreements were better between FC and BMBx than BMA and BMBx, particularly in small lymphoid neoplasms.*

Conclusion: *BMA tended to overdiagnose lymphoid neoplasms and could not accurately differentiate subtypes of B-cell and T-cell neoplasms. FC correlated more with BMBx and had less false positivity than BMA. Further utilzation of broader FC markers may help to improve the diagnostic capability and sensitivity of FC.*

Keywords: *Lymphoid Neoplasms, Flow cytometry, Immunopheno typing, Bone marrow aspiration, Bone marrow biopsy*

J Med Assoc Thai 2013; 96 (Suppl. 2): S210-S215
Full text. e-Journal: *http://jmat.mat.or.th*

Lymphoid neoplasms are the largest group of hematologic malignancies, consisting of several subtypes of B-cell, T/NK cell neoplasms and other rarer disorders[1-3]. Among B-cell lymphoid neoplasms, diffuse large B-cell lymphoma (DLBCL) is the most common subtype while peripheral T-cell lymphoma (PTCL) is the major subtype of T/NK lymphoid neoplasms[4,5]. The outcomes of patients depend on the subtypes of lymphoid neoplasms as well as the disease stages as classified into stage I-IV. Patients with an early stage (I-II) have a better prognosis than those with an advanced stage (III-IV). When bone marrow (BM) becomes involved or infiltrated by lymphoid neoplastic cells, the disease stage is recognized as stage IV and usually associates with a poor prognosis in most types of lymphoid neoplasms[6-9].

The frequency of BM involvement by lymphoid neoplasms is quite variable and depends on the disease subtype[6-9]. Indolent lymphoid neoplasm such as follicular lymphoma has a tendency to invade BM at the initial outset while an aggressive DLBCL more frequently involves BM during the course of disease[4-9]. In some cases, BM failure is the presenting symptom, thus necessitating BM studies as the first investigation of choice. BM studies are thus essential not only for the initial staging of lymphoid neoplasms but also for the follow-up of the patients. The main elements of BM studies comprise BM aspiration (BMA) and BM biopsy (BMBx). BMA is operator-dependent and subjective in determining the involvement of lymphoid neoplasms while BMBx results can be improved by utilization of immunochemical markers to

Correspondence to:
Auewarakul C, Division of Hematology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, 2 Prannok Road, Bangkoknoi, Bangkok 10700, Thailand.
Phone: 0-2419-4449, Fax: 0-2411-2012
E-mail: chirayu.aue@mahidol.ac.th

differentiate subtypes of lymphoid cells and careful assessment of a broader area of core BM[8,9].

In the present study, the authors set out to determine if flow cytometry (FC) could be used to accurately diagnose lymphoid neoplasms in the BM, either alone or in conjunction with BMA and BMBx. FC was performed in all cases clinically suspected of lymphoid neoplasms and the results were compared to BMA and BMBx results that were obtained through the hospital medical records system.

Material and Method

Patient samples

The present study was a retrospective analysis approved by the Ethical Committee for Human Research, Faculty of Medicine Siriraj Hospital, Mahidol University. BM samples from 283 patients with a presumptive clinical diagnosis of lymphoid neoplasms were studied by FC. BMA was routinely read by hematologists and BMBx was analysed by hematopathologists. The results of BMA and BMBx were retrieved through the hospital medical records system.

FC analysis of BM

BM samples were labeled with the following panel of monoclonal antibodies (BD Biosciences, CA, USA): CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11c, CD16, CD19, CD20, CD22, CD23, CD25, CD38, CD45, CD56, CD57, CD79b, CD103, FMC7, kappa and lambda. After a 15-minute incubation at room temperature in the dark, cells were lysed in FACS lysing buffer 10 minutes and washed with phosphate-buffered saline (PBS). CD45 and side scatter (SSC) gates were used to select lymphocyte windows for multiparameter flow cytometric analysis (FACScalibur; Becton Dickinson, San Jose, CA, USA). A threshold of 30% or more lymphocytes in the total events of flow cytometric gating has been used in the diagnostic criterion for NHL. A membrane marker was considered positive when more than 20% of the cells expressed it[10].

Statistical analysis

Data were described as median and range or mean and standard deviation when continuous and as absolute and relative frequency when categorical. Inter-method results were compared using the pathologists' BMBx results as the gold standard. Agreements in the diagnosis of lymphoid neoplasms between BMBx and FC and agreements between BMBx and BMA were evaluated by kappa value.

Results

Of 283 samples sent for FC analysis with a clinical suspicion of lymphoid neoplasms, 94 cases were positive for lymphoid neoplasms by BMBx (33%), 62 cases by FC (22%) and 118 cases by BMA (42%) (Table 1). Among the 94 BMBx-positive cases, 55% were positive by FC, 66% positive by BMA, 73% positive by FC or BMA and 27% were negative for both FC and BMA. Of 189 BMBx-negative cases, 8 cases were positive by FC (4%), 56 cases positive by BMA (30%), and 59 cases positive by FC or BMA (31%). All three investigations (BMBx+ BMA+ FC+) were present in 45/94 cases (47.87%).

Using BMBx as the gold standard, the sensitivity of FC, BMA and FC/BMA to diagnose lymphoid neoplasms was 55%, 66% and 73%, respectively, while the specificity of FC, BMA and FC/BMA was 96%, 70%, and 69%, respectively. The positive predictive value (PPV) and negative PV (NPV) for FC and BMA was 87% and 53%, respectively.

Table 2 shows the details of 94 cases with positive BMBx. FC correlated with BMBx with respect

Table 1. Comparison of positivity and negativity of FC and BMA to BMBx

	BMBx-positive[b] for lymphoid neoplasm (94)	BMBx-negative for lymphoid neoplasm (189)
FC positive	52 (55%)	8 (4%)
FC negative	42 (45%)	181 (96%)
BMA positive[a]	62 (66%)	56 (30%)
BMA negative	32 (34%)	133 (70%)
BMA positive[a] or FC positive	69 (73%)	59 (31%)
BMA and FC both negative	25 (27%)	130 (69%)

[a] Positive or suspicious abnormal cells as remarked by reading hematologists in the final reports
[b] Positive abnormal cells as remarked by reading pathologists in the final reports

Table 2. Detailed results of diagnosis of lymphoid neoplasms by BMBx, BMA, and FC using BMBx as the gold standard

Patient No.	BMBx diagnosis	BMA diagnosis	FC diagnosis
1-2	CLL	CLL	CLL
3	CLL	FL	CLL
4	CLL	Small B- cell neoplasm	Small B- cell neoplasm
5	CLL	Non-diagnostic	Small B- cell neoplasm
6	CLL	Normal	Non-diagnostic
7	MCL	MCL	MCL
8	MCL	CLL	MCL
9-12	MCL	Lma	MCL
13-14	MCL	No evidence of Lma	MCL
15	MCL	Lma	No evidence of Lma
16	MCL	No evidence of Lma	No evidence of Lma
17-18	SMZL	SMZL	SMZL
19	SMZL	Non-diagnostic	SMZL
20	SMZL	Non-diagnostic	Non-diagnostic
21	HCL	HCL	HCL
22	HCL	Lma	HCL
23	HCL	Non-diagnostic	No evidence of Lma
24-25	FL	Lma	FL
26-28	FL	Lma	Small B- cell neoplasm
29	FL	Non-diagnostic	Small B- cell neoplasm
30-32	FL	Lma	No evidence of Lma
33	FL	FL	No evidence of Lma
34-35	FL	No evidence of Lma	No evidence of Lma
36-39	Small B-cell neoplasm	Lma	CLL
40-44	Small B-cell neoplasm	Lma	SMZL
45	Small B-cell neoplasm	Inadequate specimen	HCL
46	Small B-cell neoplasm	Multiple myeloma	Plasma cell myeloma
47-51	Small B-cell neoplasm	Lma	Small B- cell neoplasm
52-55	Small B-cell neoplasm	No evidence of Lma	No evidence of Lma
56	Large B-cell lymphoma	PTCL	B lymphoid neoplasm
57	Large B-cell lymphoma	No evidence of Lma	B lymphoid neoplasm
58	Large B-cell lymphoma	Lma	B lymphoid neoplasm
59-65	Large B-cell lymphoma	Lma	No evidence of Lma
66-72	Large B-cell lymphoma	No evidence of Lma	No evidence of Lma
73	Malignant lymphoma	Lma	Lma
74-75	Malignant lymphoma	Lma	No evidence of Lma
76	Malignant lymphoma	No evidence of Lma	No evidence of Lma
77-78	T-cell lymphoma	Lma	T-cell lymphoma
79-81	T-cell lymphoma	Lma	No evidence of Lma
82-84	T-cell lymphoma	No evidence of Lma	No evidence of Lma
85-86	Burkitt's lymphoma	Burkitt's lymphoma	Burkitt's lymphoma
87	Burkitt's lymphoma	Burkitt's lymphoma	B lymphoid neoplasm
88	Burkitt's lymphoma	No evidence of Lma	No evidence of Lma
89-90	Lymphoplasmacytic Lma	Lymphoplasmacytic Lma	Lymphoplasmacytic Lma
91	Lymphoplasmacytic Lma	Lymphoplasmacytic Lma	B lymphoid neoplasm
92-94	Lymphoplasmacytic Lma	No evidence of Lma	No evidence of Lma
95-283	No evidence of Lma (189)	No evidence of Lma (133)	No evidence of Lma (181)

CLL, Chronic lymphocytic leukemia; HCL, hairy cell leukemia; FL, follicular lymphoma; MCL, mantle cell lymphoma; SMZL, splenic marginal zone lymphoma; Lma, lymphoma

to subtypes of lymphoid neoplasms while BMA tended to give an impression of "suspicious for lymphoma" without giving the subtype of the disease. FC failed to diagnose DLBCL and T-cell lymphoma as compared to small B-cell lymphoid neoplasms.

Table 3 shows the correlation between FC/BMBx and BMA/BMBx with respect to each subtype of lymphoid neoplasms found in this study. The highest proportion of concordant cases was noted in small lymphoid neoplasms. In mantle cell lymhpoma (MCL), FC agreed with BMBx more than BMA with BMBx. In "malignant lymphoma" of unspecified type, BMA agreed with BMBx more than FC with BMBx.

Discussion

BM involvement by lymphoid neoplasms varies depending upon subtypes. In some entities, the boundary between lymphoid neoplasm invading into the BM (i.e. stage IV disease) and lymphoid neoplasm originating in the BM (i.e. leukemia) is blurred[1-3]. In the present study, various subtypes of lymphoid neoplasms were identified such as CLL, MCL, FL, MZL, Burkitt's lymphoma and lymphoplasmacytic lymphoma. Overall, BMA significantly diagnosed more cases of lymphoid neoplasms than FC and BMBx. However, a large number of BMA-positive cases turned out to be normal by BMBx and many of them were also negative by FC. FC appeared to match more with BMBx with respect to specific subtypes of lymphoid neoplasms, particularly small B-cell neoplasms, CLL, MCL and MZL. Nevertheless, it is of interest to note that FC failed to diagnose many cases of large B-cell and T-cell lymphoma whereas BMA could diagnose more cases of such diseases although without specific lineage assignment. This could be due to the fact that many malignant B-cells and T-cells may be mixed among normal B-cells and T-cells, making it difficult to separate them from one another, particularly if the malignant cells do not exhibit other unique characteristics by FC[10-15].

Although the sensitivity of FC was slightly lower than BMA (55% vs. 66%), the specificity of FC was much higher than BMA (96% vs. 70%), indicating the value of FC for confirming the negative BMBx results. If FC and BMA were used together, the sensitivity increased to 73% but the specificity was then compromised. The agreement of the three investigations of 48% in our study was comparable to 56% in the previous study by Sah SP et al[15]. The agreement between FC and BMBx of about 60% in our study was also comparable to 69% (27/39) in their study.

The authors' data demonstrated that BMA tended to over diagnose lymphoid neoplasms when BMBx was used as the gold standard. FC, on the other hand, tended to under diagnose lymphod neoplasms, particularly large cell types. Overall, BMBx correlated better with FC than with BMA in the diagnosis of lymphoid neoplasms. Further modifications of FC markers may help to improve the sensitivity of FC for diagnosis of lymphoid neoplasms. TCR-alpha/beta and TCR-gamma/delta can also be utilized to improve the diagnosis of T-cell neoplasms.

In conclusion, FC can be utilized in conjunction with BMBx to diagnose BM involvement by lymphoid neoplasms. FC is better than BMA in terms

Table 3. Correlation between FC, BMA and BMBx in the diagnosis of subtypes of lymphoid neoplasms

	BMBx (%)	BMA (%)	Kappa value	p-value	FC (%)	Kappa value	p-value
CLL	6.4	3.2	0.420	< 0.001	7.4	0.422	< 0.001
MCL	10.6	1.1	0.166	0.004	8.5	0.877	< 0.001
SMZL	4.3	2.1	0.657	< 0.001	8.5	0.470	< 0.001
HCL	3.2	1.1	0.492	< 0.001	3.2	0.656	< 0.001
FL	12.8	2.1	0.110	0.111	2.1	0.259	< 0.001
Small B- cell neoplasm	21.3	0	-*	-*	11.7	0.202	0.037
Large B-cell lymphoma	18.1	0	-*	-*	3.2	0.260	< 0.001
Malignant Lma	4.3	3.2	0.852	< 0.001	1.1	0.390	< 0.001
T-cell lymphoma	8.5	0	-*	-*	2.1	0.379	< 0.001
Burkitt's lymphoma	4.3	3.2	0.379	< 0.001	2.1	0.657	< 0.001
Lymphoplasmacytic lymphoma	6.4	3.2	0.652	< 0.001	2.1	0.484	< 0.001

*different results from BMBx

of false positivity and specificity. BMA should not be used alone to diagnose BM involvement by lymphoid neoplasms. The sensitivity of FC should be improved by further modifications of immunophenotypic markers for malignant B-cells and T-cells.

Acknowledgement

The authors wish to thank the staff of the Division of Hematology, Department of Medicine, Faculty of Medicine Siriraj Hospital, for excellent care of the patients in the present study and the staff of Department of Pathology, Faculty of Medicine Siriraj Hospital for their kind reports of routine BMBx results.

Potential conflicts of interest

None.

References

1. Evans LS, Hancock BW. Non-Hodgkin lymphoma. Lancet 2003; 362: 139-46.
2. Jaffe ES. The 2008 WHO classification of lymphomas: implications for clinical practice and translational research. Hematology Am Soc Hematol Educ Program 2009; (1): 523-31.
3. Campo E, Swerdlow SH, Harris NL, Pileri S, Stein H, Jaffe ES. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. Blood 2011; 117: 5019-32.
4. Intragumtornchai T, Wannakrairoj P, Chaimongkol B, Bhoopat L, Lekhakula A, Thamprasit T, et al. Non-Hodgkin's lymphomas in Thailand. A retrospective pathologic and clinical analysis of 1391 cases. Cancer 1996; 78: 1813-9.
5. Sukpanichnant S. Analysis of 1983 cases of malignant lymphoma in Thailand according to the World Health Organization classification. Hum Pathol 2004; 35: 224-30.
6. Kumar S, Rau AR, Naik R, Kini H, Mathai AM, Pai MR, et al. Bone marrow biopsy in non-Hodgkin lymphoma: a morphological study. Indian J Pathol Microbiol 2009; 52: 332-8.
7. Gomyo H, Shimoyama M, Minagawa K, Yakushijin K, Urahama N, Okamura A, et al. Morphologic, flow cytometric and cytogenetic evaluation of bone marrow involvement in B-cell lymphoma. Haematologica 2003; 88: 1358-65.
8. Sukpanichnant S, Srisook K, Chormanee N. Determination of marrow involvement in non-Hodgkin's lymphoma by immunocytochemical studies. Siriraj Hosp Gasz 2001; 53: 474-80.
9. Arber DA, George TI. Bone marrow biopsy involvement by non-Hodgkin's lymphoma: frequency of lymphoma types, patterns, blood involvement, and discordance with other sites in 450 specimens. Am J Surg Pathol 2005; 29: 1549-57.
10. Craig FE, Foon KA. Flow cytometric immunophenotyping for hematologic neoplasms. Blood 2008; 111: 3941-67.
11. Swart GJ, Wright C, Brundyn K, Mansvelt E, du Plessis M, ten Oever D, et al. Fine needle aspiration biopsy and flow cytometry in the diagnosis of lymphoma. Transfus Apher Sci 2007; 37: 71-9.
12. Merli M, Arcaini L, Boveri E, Rattotti S, Picone C, Passamonti F, et al. Assessment of bone marrow involvement in non-Hodgkin's lymphomas: comparison between histology and flow cytometry. Eur J Haematol 2010; 85: 405-15.
13. Demurtas A, Accinelli G, Pacchioni D, Godio L, Novero D, Bussolati G, et al. Utility of flow cytometry immunophenotyping in fine-needle aspirate cytologic diagnosis of non-Hodgkin lymphoma: A series of 252 cases and review of the literature. Appl Immunohistochem Mol Morphol 2010; 18: 311-22.
14. Braylan RC. Impact of flow cytometry on the diagnosis and characterization of lymphomas, chronic lymphoproliferative disorders and plasma cell neoplasias. Cytometry A 2004; 58: 57-61.
15. Sah SP, Matutes E, Wotherspoon AC, Morilla R, Catovsky D. A comparison of flow cytometry, bone marrow biopsy, and bone marrow aspirates in the detection of lymphoid infiltration in B cell disorders. J Clin Pathol 2003; 56: 129-32.

การตรวจวินิจฉัยเนื้องอกของเซลล์ระบบน้ำเหลืองด้วยวิธีโฟลซัยโตเมทรีเปรียบเทียบกับการตรวจไขกระดูกด้วยวิธีเจาะดูดไขกระดูกและการตัดตรวจชิ้นเนื้อไขกระดูก

อรทัย พรหมสุวิชา, วยุรี สองเมือง, จิรายุ เอื้อวรากุล

***ภูมิหลัง**: เนื้องอกของเซลล์ระบบน้ำเหลืองประกอบไปด้วยชนิดย่อยหลากหลายชนิด และสามารถแพร่กระจายเข้าสู่ไขกระดูกได้บ่อย การวินิจฉัยภาวะแพร่กระจายของเซลล์มะเร็งเข้าสู่ไขกระดูก ต้องการการตรวจวินิจฉัยที่รวดเร็วและแม่นยำ เพื่อใช้ในการตัดสินการรักษาที่เหมาะสม*

***วัตถุประสงค์**: เพื่อประเมินการใช้โฟลซัยโตเมทรีในการวินิจฉัยการแพร่กระจายของเซลล์มะเร็งระบบน้ำเหลืองเข้าสู่ไขกระดูก โดยใช้การตัดชิ้นเนื้อไขกระดูกเป็นมาตรฐาน และเปรียบเทียบกับผลของการเจาะดูดไขกระดูก*

***วัสดุและวิธีการ**: สิ่งส่งตรวจจำนวน 283 ราย ซึ่งได้รับการวินิจฉัยเบื้องต้นจากแพทย์ว่าเป็นเนื้องอกของเซลล์ระบบน้ำเหลือง ได้รับการตรวจวิเคราะห์หาเซลล์มะเร็งด้วยวิธีโฟลซัยโตเมทรี และเปรียบเทียบผลของโฟลซัยโตเมทรีกับผลการเจาะดูดไขกระดูกและผลการตัดตรวจชิ้นเนื้อไขกระดูก*

***ผลการศึกษา**: ในผู้ป่วยจำนวน 283 ราย มีผู้ป่วย 94 ราย ที่ได้รับการยืนยันว่าเป็นเนื้องอกของเซลล์ระบบน้ำเหลืองด้วยวิธีการตัดตรวจชิ้นเนื้อไขกระดูก (ร้อยละ 33) ผู้ป่วยที่พบว่ามีผลการวินิจฉัยโรคตรงกันทั้งสามวิธี มีจำนวน 45 ราย (ร้อยละ 48) ผลโฟลซัยโตเมทรีให้ผลบวก 52/94 ราย (ร้อยละ 55) ในขณะที่ผลการเจาะดูดไขกระดูกให้ผลบวก 62/94 ราย (ร้อยละ 66) การตัดตรวจชิ้นเนื้อไขกระดูกให้ผลลบโดยที่โฟลซัยโตเมทรีให้ผลบวก 8/189 ราย (ร้อยละ 4) และการเจาะดูดไขกระดูกให้ผลบวก 56/189 ราย (ร้อยละ 30) โฟลซัยโตเมทรีและผลการเจาะดูดไขกระดูกให้ผลลบทั้งคู่ 25/94 ราย (ร้อยละ 27) ความจำเพาะของโฟลซัยโตเมทรีและการเจาะดูดไขกระดูกเท่ากับ ร้อยละ 96 และร้อยละ 60 ขณะที่ความไวเท่ากับ ร้อยละ 55 และร้อยละ 65 ตามลำดับ การแยกชนิดย่อยของเนื้องอกโดยใช้โฟลซัยโตเมทรีและการตัดตรวจชิ้นเนื้อไขกระดูกให้ผลตรงกันมากกว่าผลของการเจาะดูดไขกระดูกกับการตัดตรวจชิ้นเนื้อไขกระดูก โดยเฉพาะอย่างยิ่งเนื้องอกประเภทเซลล์เล็ก*

***สรุป**: การแปลผลไขกระดูกด้วยวิธีเจาะดูดมักให้ผลการวินิจฉัยโรคมะเร็งระบบน้ำเหลืองสูงกว่าความเป็นจริง และไม่สามารถแยกชนิดย่อยของบีเซลล์และทีเซลล์ได้ โฟลซัยโตเมทรีให้ผลที่ไปด้วยกันกับการตัดตรวจชิ้นเนื้อไขกระดูกโดยพยาธิแพทย์ โดยให้ผลบวกลวงน้อยกว่าผลของการอ่านไขกระดูกที่ได้จากวิธีเจาะดูด การใช้แอนติบอดีหลายชนิดร่วมกันในการวิเคราะห์ด้วยโฟลซัยโตเมทรีจะมีประโยชน์และช่วยส่งเสริมความสามารถในการวินิจฉัยและเพิ่มความไวของโฟลซัยโตเมทรี*