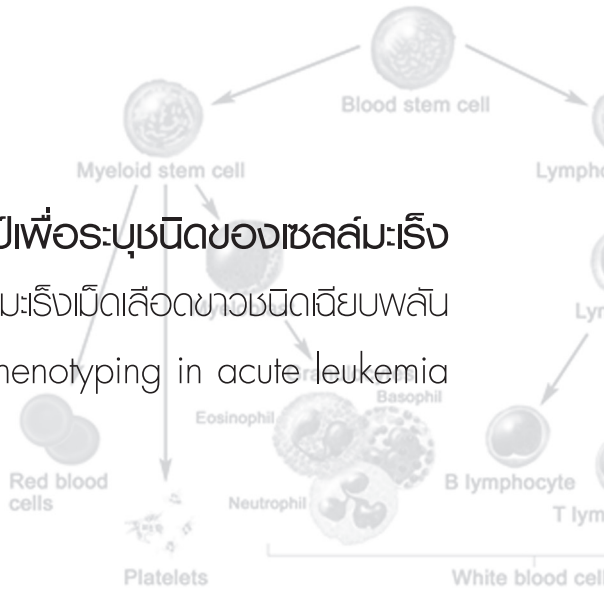

บทบาทของการตรวจวิเคราะห์อิมมูโนฟีโนทัยป์เพื่อระบุชนิดของเซลล์มะเร็ง

ในผู้ป่วยโรคมะเร็งเม็ดเลือดขาวชนิดเฉียบพลัน

Lineage assignment role of immunophenotyping in acute leukemia

นพพร จึงพิชาญวณิชย์[1*] *Nopporn Juengpichanvanich*[1*]
ชรินทร์ ยาอินทร์[2] *Charin Ya-in*[2]
เลิศลักขณา ภู่พัฒน์[2] *Lertlakana Bhoopat*[2]
ลลิตา นรเศรษฐ์ธาดา[3] *Lalita Norasetthada*[3]

[1] *กลุ่มงานพยาธิวิทยาคลินิก โรงพยาบาลนครพิงค์ เชียงใหม่*
[2] *แผนกพยาธิวิทยาคลินิก ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่*
[3] *หน่วยโลหิตวิทยา ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่*

[1] *Department of Clinical Pathology, Nakornping Hospital, Chiang Mai, Thailand*
[2] *Division of Clinical Pathology, Department of Pathology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand*
[3] *Hematology Division, Department of Internal Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand*

[*] *ผู้รับผิดชอบบทความ*
* *Corresponding author*

บทคัดย่อ

วัตถุประสงค์:

เพื่อประเมินบทบาทของผลการตรวจอิมมูโนฟีโนทัยป์ด้วยเทคนิคโฟล ซัยโทเมตรีในการระบุชนิดของเซลล์มะเร็งในผู้ป่วยโรคมะเร็งเม็ดเลือดขาวชนิดเฉียบพลัน

วัสดุและวิธีการ:

ศึกษาผู้ป่วยโรคมะเร็งเม็ดเลือดขาวชนิดเฉียบพลันจำนวน 100 รายที่ได้รับการตรวจวินิจฉัยที่โรงพยาบาลมหาราชนครเชียงใหม่ คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ ในระหว่างปี พ.ศ. 2553 โดยเปรียบเทียบผลอิมมูโนฟีโนทัยป์จากการตรวจด้วยเทคนิคโฟล ซัยโทเมตรี กับการตรวจสัณฐานวิทยา

ผลการศึกษา:

วิธีทางสัณฐานวิทยาสามารถระบุชนิดของเซลล์มะเร็งที่ตรวจพบในผู้ป่วยได้ครบทั้งหมด แต่พบว่าการตรวจอิมมูโนฟีโนทัยป์ด้วยเทคนิคโฟล ซัยโทเมตรีให้ผลต่างจากการตรวจด้วยวิธีทางสัณฐานวิทยาอย่างมีนัยสำคัญ ($p<0.001$) จำนวนทั้งหมด 16 ราย (16%) โดยเปลี่ยนจากชนิดลิมฟอยด์เป็นชนิดมัยลอยด์จำนวน 8 ราย และจากชนิดมัยลอยด์เป็นชนิดลิมฟอยด์จำนวน 8 ราย

สรุป:

ผลการศึกษาครั้งนี้ได้แสดงให้เห็นถึงประโยชน์และช่วยยืนยันถึงความจำเป็นในการวิเคราะห์ผลอิมมูโนฟีโนทัยป์ด้วยเทคนิคโฟล ซัยโทเมตรี เพื่อการรักษาที่ถูกต้องเหมาะสมของผู้ป่วยโรคมะเร็งเม็ดเลือดขาวชนิดเฉียบพลัน

คำหัส: *การตรวจวิเคราะห์อิมมูโนฟีโนทัยป์ โรคมะเร็งเม็ดเลือดขาวชนิดเฉียบพลัน การระบุชนิดของเซลล์มะเร็งเม็ดเลือดขาว โฟล ซัยโทเมตรี*

Abstract

Objective:

To assess the role of routine multiparameter flow cytometry, for lineage assignment of leukemic cells in patients with acute leukemia.

Materials and Methods:

One hundred cases of adult and children patients diagnosed with acute leukemia admitted at Maharaj Nakorn Chiang Mai Hospital during 2010 were reviewed. Bone marrow samples were immunophenotyped by multiparameter flow cytometry compared to morphology.

Results:

In our retrospective analysis of 100 cases, morphology established lineage in all cases (100%). However, immunophenotyping significantly indicated lineage assignment difference from morphology in 16 cases (16%) with $p<0.001$. The first group of 8 cases was reported to be lymphoid from previously reported myeloid. The second group of 8 cases was confirmed to be myeloid from previously reported lymphoid.

Conclusion:

The results addressed the significant benefit and supported routine application of immunophenotyping in the diagnosis of acute leukemia.

Keywords: *immunophenotyping, lineage assignment, acute leukemia, multiparameter flow cytometry*

Introduction

Assignment of lineage is very important in the diagnosis of acute leukemia, as treatments for acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) are different. Identification of myeloid and lymphoid lineage may be recognized based on cellular morphology, cytochemical staining, and immunophenotyping (expression of lineage-specific antigens). The diagnosis and classification of hematopoietic neoplasms have greatly improved from the general application of immunophenotyping during the past two decades. The identification of hematopoietic neoplasms by their association to normal hematopoietic lineages and stages of differentiation is a basic datum of current classification systems, such as the World Health Organization (WHO) classification. This information is largely provided by immunophenotyping and has resulted in the addition of immunophenotypic data into the definition of many hematopoietic neoplasms. At present, the certain diagnosis and classification of hematopoietic neoplasms cannot be confidently made without immunophenotypic data.[1,2,3] Myeloid antigen expression in acute leukemia that is morphologically and cytochemically undifferentiated helps to identify the French–American–British (FAB) M0 subtype of AML in the FAB classification or minimally differentiated AML in the World Health Organization (WHO) classification.[4,5] At Maharaj Nakorn Chiang Mai Hospital, diagnostic evaluation of acute leukemia has routinely included immunophenotyping by multiparameter flow cytometry, in addition to morphologic results. We, therefore retrospectively analyzed the lineage assignment role of immunophenotyping in acute leukemia compared to morphology.

Materials and Methods

Patients: One hundred cases of acute leukemia diagnosed continuously in adults and children at Maharaj Nakorn Chiang Mai Hospital during the year 2010 were reviewed.

Morphology: Bone marrow aspirate smears were stained with Wright-Giemsa for morphological analysis. Morphological results were compared with the immunophenotypic results.

Immunophenotyping: Bone marrow specimens were

immunophenotyped by multiparameter flow cytometry in the Laboratory of Flow Cytometry at Maharaj Nakorn Chiang Mai Hospital. Cell suspensions were stained with multiple panels of three monoclonal antibodies labeled with fluorescein isothiocyanate (FITC), phycoerythrin (PE), and peridinin chlorophyll protein (PerCP). Immunophenotypic data were acquired on a FACSCanto II flow cytometer (BD Biosciences). Data were analyzed using BD FACSDiva software. Antigen-positivity was defined by reactivity above IgG isotype matched controls of leukemic cells. Intracellular antigen was not done in this retrospective studies, therefore analysis were based on surface expression. Gating was done by the combination of FSC versus SSC and CD45 versus SCC dot plots.

Statistical analysis: Proportions of acute leukemia was expressed using descriptive statistics. Chi-square was used for the analysis of categorical data by SPSS program version 11.5 for Windows.

Results

One hundred cases of acute leukemia were diagnosed at Maharaj Nakorn Chiang Mai Hospital during the year 2010. Morphological analysis established lineage in all patients (100%). Myeloid and lymphoid lineage was identified in 61 and 39 patients respectively. Immunophenotyping changed the lineage assigned based on morphology in 16 cases (16%); 8 changed from lymphoid to myeloid, and 8 from myeloid to lymphoid. All cases with acute promyelocytic leukemia (APL) can be distinguished based on morphology (100%) which has been confirmed by immunophenotyping.

Among 39 patients with ALL based on morphology, immunophenotyping demonstrated lymphoid immunophenotypes in 31 patients (26 cases of B-ALL and 5 cases of T-ALL), but demonstrated myeloid immunophenotypes in 8 patients. These 8 cases of myeloid immunophenotypes had present bright expression of CD33 and/or CD13, and absence of lymphoid markers with the exception of aberrant dim expression of CD19 (2 cases), CD4 (1 case), CD56 (2 cases), and CD20 (2 cases).

Among 61 patients with AML based on morphology, 53 patients had myeloid immunophenotypes, but 8 patients had lymphoid immunophenotypes (7 cases of B-ALL and 1 case of T-ALL). B-ALL was identified by the HLA-DR, CD34, CD19, and CD22 phenotype in all 7 cases, with aberrant dim expression of CD4 (1 case) and CD33 (1 case). T-ALL was identified by the HLA-DR, CD34, CD3, and CD7 phenotype.

There were significantly different between morphological analysis and flow cytometric analysis ($p<0.001$ by Pearson Chi-square test). Results were shown in Table 1 and 2.

Table 1 Diagnosis of acute leukemia based on morphology and immunophenotyping

			Flow cytometric analysis			Total
			AML	B-ALL	T-ALL	
Morphological analysis	AML	Count	45	7	1	53
		% within Morphology	84.9%	13.2%	1.9%	100%
	ALL	Count	8	26	5	39
		% within Morphology	20.5%	66.7%	12.8%	100%
	APL (M3)	Count	8	0	0	8
		% within Morphology	100%	0%	0%	100%
Total		Count	61	33	6	100
		% within Morphology	61%	33%	6%	100%

Table 2 The difference between morphological analysis and flow cytometric analysis

			Morphological analysis		Total
			AML	ALL	
Flow cytometric analysis	AML	Count	53	8	61
		% within Flow cytometry	86.9%	13.1%	100%
	ALL	Count	8	31	39
		% within Flow cytometry	20.5%	79.5%	100%
Total		Count	61	39	100
		% within Flow cytometry	61%	39%	100%

Discussion

Flow cytometry is now considered to be an important component of immunophenotyping in hemato-pathology. Because plentiful of commercially available monoclonal antibodies against clusters of differentiation (CD) molecules with conjugated fluorochromes, advanced flow cytometry hardwares and softwares, improved gating strategies, and multiparameter analysis have made flow cytometry a valuable method for immunophenotyping. Gating procedure is extremely important in separating non-blast cells from blast cells. Proper gating is a crucial step in data interpretation of the results in flow cytometry.[6]

Lineage assignment is very important for proper therapy for acute leukemia, as treatment regimens for ALL and AML differ clearly. The diagnosis and classification of hematopoietic neoplasms is enhanced by the ability to accurately identify abnormal cells, assign lineage and determine stage of maturation, principally for chronic lymphoproliferative disorders and acute leukemias. Flow cytometry is now extensively used for this purpose. [3,7,8]

In our retrospective analysis of routine immunophenotyping in the diagnosis of 100 cases of acute leukemia, immunophenotyping was essential for lineage assignment in 16 cases, by corrected the lineage that was assigned from morphology. The frequency which immunophenotyping changed the lineage assigned from morphology was 16%. There were significantly different between morphological analysis and flow cytometric analysis (p<0.001).

The original FAB classification was based on morphological and cytochemical findings to classify leukemias.[9,10] The EGIL and WHO recommended an immunological classification of leukemias with development of scoring system to classify between various subtypes of leukemia.[3,11,12] The crucial role of flow cytometry in acute leukemia is classification. Classification is mainly about lineage assignment and association with normal stage of maturation. The accurate lineage determination between myeloid and lymphoid lineage of leukemic cells is critical for the proper therapeutic regimens.

The discrepancy between morphology and immunophenotypic findings were range from 7% to 10% in the other previous studies.[13,14] In this retrospective analysis of routine immunophenotyping in 100 cases of acute leukemia, immunophenotyping was essential for lineage correction in 16% of cases (p<0.001). These data supported an augmentation for routine utilization of immunophenotyping in the diagnostic method of acute leukemia.

References

1. Vardiman JW, Arber DA, Brunning RD. Acute myeloid leukaemia (AML) and related precursor neoplasms. In: Steven HS, Elias C, Nancy LH, *et al*, eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press; 2008: 110-47.
2. Borowitz MJ, Chan JKC. Precursor lymphoid neoplasms. In: Steven HS, Elias C, Nancy LH, *et al*, eds. WHO classification of tumours of hematopoietic and lymphoid tissues. 4th ed. Lyon, France: IARC, 2008: 167-78.
3. Brent LW, Michael JB. The Flow Cytometric Evaluation of Hematopoietic Neoplasia. In: Mcpherson RA, Pincus MR, eds. Henry's clinical diagnosis and management by laboratory methods.21th ed. Burlington, Massacheusetts: Elsevier, 2007: 599-617.
4. Bennett JM, Catovsky D, Daniel MT, *et al*. Proposal for the recognition of minimally differentiated acute myeloid leukaemia (AML-MO). Br J Haematol 1991; 78: 325–9.
5. Arber DA, Brunning RD, Orazi A, *et al*. Acute myeloid leukemia, not otherwise specified. In: Steven HS, Elias C, Nancy LH, *et al*, eds. WHO classification of tumours of hematopoietic and lymphoid tissues. 4th ed. Lyon: IARC, 2008: 130-9.
6. Faramarz N. Principles of Immunophenotyping. In: Faramarz N, Nagesh R, Wayne WG. Hemato pathology, 1st ed. Burlington, Massacheusetts: Elsevier, 2008: 27-55.
7. Dan LL. Malignancies of Lymphoid Cells. In: Fauci AS, Brauwald E, Kasper DL, et al, eds. Harrison's principles of internal medicine, 17th ed. New York: McGraw-Hil, 2008: 687-99.
8. Mier W, John CB, Clara DB. Acute and Chronic Myeloid Leukemia. In: Fauci AS, Brauwald E, Kasper DL, *et al*, eds. Harrison's principles of internal medicine, 17th ed. New York: McGraw-Hil, 2008: 677-86.
9. Bennett JM, Catovsky D, Daniel MT, *et al*: Proposals for the classification of the acute leukaemias. French–American–British (FAB) Cooperative Group. Br J Haematol 1976; 33: 451-8.
10. Robert EH, Naif ZAJ. Leukocytic Disorders. In: Mcpherson RA, Pincus MR, eds. Henry's clinical diagnosis and management by laboratory methods, 21th ed. Burlington, Massacheusetts: Elsevier, 2007: 545-98.
11. Bene MC, Castoldi G, Knapp W, *et al*. Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). Leukemia 1995; 10: 1783–6.
12. Thalhammer SR, Mitterbauer G, Simonitsch I, *et al*. The immunophenotype of 325 adult acute leukemias: Relationship to morphologic and molecular classification and proposal for a minimal screening program highly predictive for lineage discrimination. Am J Clin Pathol 2002; 117: 380–9.
13. Misbah Q, Maurice B, Carleton CS, *et al*: Routine immunophenotyping in acute leukemia: role in lineage assignment and reassignment. Cytometry B 2006; 70: 329-34.
14. Peter BN, Praniti S, George PB, *et al*: Classifying acute leukemia by immunophenotyping: a combined FAB-immunologic classification of AML. Blood 1986; 68(6): 1355-62.