Original Article

Cytochemistry and Multi-color Flow Cytometric Immunophenotype for Diagnosis of Childhood Acute Leukemia

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Abstract : *The combined techniques of cytochemistry and flow cytometry are currently used for definite diagnosis and risk stratification of childhood acute leukemias. The aim of the study was to evaluate the usefulness of these methods for establishing diagnosis of childhood acute leukemias. This was a retrospective study of children with newly diagnosed acute leukemia. One hundred and fourteen children, 75 males and 39 females, with a median age of 6 years (0-15 years) were included in the study. Blood counts showed anemia (Hb <10 g/dL) and thrombocytopenia (platelets <100 x10^9/L) in 71% and 80%, respectively. Most of the patients (66%) presented with significant blast cells (>25%) in peripheral blood. Acute myeloid leukemia (AML) represented 26.3% (30) of cases. FAB M2 cell type was the most common AML. Myeloperoxidase enzyme reactivity was strong in M1 to M3. CD13 and CD33 were the most useful markers for diagnosis of AML. Aberrant expression of lymphoid antigens was seen in 47%, in which CD7 was the most frequent. Acute lymphoblastic leukemia (ALL) was diagnosed in 84 (73.7%) cases. Flow cytometry detected B lineage in 85.7% and T lineage in 14.3%. The early pre B ALL (CD10+) was found in 62 cases (86.1%). Expression of myeloid antigens including CD11b, CD13, CD15, or CD33 was detected in 14% of B lineage ALL. This study demonstrated that cytochemistry and flow cytometry were successfully used for diagnosis and subtype classification of childhood acute leukemias.*

Key Words : ● *Acute leukemia* ● *Cytochemistry* ● *Flow cytometry*

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Introduction

Acute leukemia is the most common pediatric malignancy. The disease arises from the dysregurated clonal expansion of immature lymphoid or myeloid progenitor cells that are blocked at a particular stage of differentiation.[1] The diagnosis of acute leukemia traditionally has been based on the evaluation of peripheral blood counts, bone marrow morphology and cytochemistry staining. Diagnosis uniformity has been enhanced with introduction and later modification of the French-American-British (FAB) classification system, which is based upon morphologic and cytochemical characteristic and provides as a standardization of criteria for the subclassification of myeloid and lymphoid leukemias.[2] However, it is still insufficient for the classification of many leukemias, as morphologic characteristic may overlap and cytochemistry may be negative or equivocal. In most cases of poorly differentiated leukemias, the lineage cannot be definitely diagnosed by these methods. Advances in flow cytometry technology and the available

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monoclonal antibodies are substantially helpful to assess myeloid and lymphoid antigens by cytometry. In acute myeloid leukemia (AML), immunophenotyping is the most important in distinguishing poorly differentiated case from acute lymphoblastic leukemia (ALL) and in characterizing a few AML subsets.[3] For ALL, the immunophenotyping categories are particularly important because they identify distinctive treatment protocol and prognostic groups.[4]

The purpose of this study was to evaluate 114 consecutive cases of childhood acute leukemias using a multiparameter approach consisting of a combination of Wright-Giemsa smear and cytochemistry. We have concentrated on an improved definition of the cellular specific phenotype in ALL and AML using a large panel surface and intracellular antigens.

Materials and Methods

Patients and specimen collection

We studied data of patients with newly-diagnosed acute leukemia at the department of Pediatrics, Srinagarind Hospital, Faculty of Medicine, Khon Kaen University during 2006 and 2007. Either bone marrow aspirate or peripheral blood was immediately transported in EDTA or sodium heparin tubes to the flow cytometry laboratory. The minimum blast count in any specimen was 30%.

Morphology and cytochemical analysis

All specimens were obtained and prepared for morphologic examination using standard techniques. Bone marrow aspirate smears and peripheral blood specimens (when available) were air dried and stained with Wright-Giemsa technique and examined under light microscopy. Peripheral blood and bone marrow cells were routinely stained for the presence of myeloperoxidase (MPO), periodic acid-Schiff (PAS), naphthol AS-D chloroacetate esterase (CAE) and alpha-naphthyl butyrate esterase (ANBE) using a standard procedure. At least 200 cells were counted to determine the percentage of positive cells. Stain positivity for MPO and PAS was determined if the reactivity was present in 3% of blast cell population but CAE and NBE required >20% positivity.

Flow cytometry analysis

Red blood cell lysis after immunofluorescent staining method was used in this study. Briefly, cell were stained by direct immunofluorescence using 10 μL of conjugate monoclonal antibodies (MoAbs) added to 100 μL of whole blood or bone marrow. It was then gently mixed and incubated for 10 minutes at room temperature in the dark. Sample was washed with 2% bovine serum albumin in phosphate buffer saline (PBSA) and centrifuged at 500x g for 5 minutes. The supernatant was removed and 500 μL of lysing agent (Optilyse C, Immunotech, France) were added in each sample. It was then vortexed gently and incubated for 10 minutes at room temperature. Subsequently, 3 ml of 2% PBSA were added to stop lysis and centrifuged again to remove supernatant. For the detection of cytoplasmic or nuclear antigen, Intraprep permeabilization reagent kit (Immunotech, France) was performed to permeabilize the cells. Finally, 500 μL of 0.5% paraformaldehyde in PBS were added and the samples analyzed by Epics XL flow cytometry.

The cells were stained with various combinations of fluorescein isothiocyanate (FITC)-, phycoerythrin (PE)-, and phycoerythrin-cyanine 5 (PC5)-labeled monoclonal antibodies against the following antigens (Immunotech, France): CD2, surface CD3, CD5, CD7, CD10, CD11b, CD13, CD14, CD15, CD19, CD20, CD22, CD33, CD34, CD41, CD56, CD61, CD117, HLA-DR and TdT. In several cases, additional antigens were used, including CD4, CD8, cytoplasmic (c) CD3, cCD79a, cIgM, glycophorin A, T cell receptor (TCR) α/β and TCR γ/δ.

Three or four color flow cytometric immunophenotype was performed on the Coulter Epics XL cytometer (Beckman-Coulter, Miami, FL) by collecting 15,000 ungated list mode events, selecting an appropriate blast gate on the combination of forward and side scatter, and analyzing cells with the most appropriate blast gate. Many of the cases were gated on CD45 dim versus side scatter to isolate the blast population and 15,000 list mode events were collected on the blast gate. A positive

reaction was considered if at least 20% of the leukemic cells revealed higher fluorescence intensities than cell stained with the isotypic Ig-FITC/PE-control antibody as proposed by European group for the immunological characterization of leukemias (EGIL).[5]

Results

Demographic, clinical and laboratory findings

The main characteristics of childhood acute leukemias patients evaluated in this study at diagnosis are summarized in Table 1. One hundred and fourteen children (75 males and 39 females) with a median age of 6 years (range, 0-15 years) were included in the study. The blood counts showed anemia (Hb<10g/dL) and thrombocytopenia (platelets <100 x10^9/L) in 71% and 80%, respectively. Thirty three percent of cases presented with hyperleukocytosis (WBC>50 x 10^9/L). Most of the patients (66%) presented with significant numbers of blast cells (>25%) in peripheral blood.

Acute Lymphoblastic Leukemia (ALL)

Morphology and Cytochemistry: Cytochemistry profile is shown in Table 2. ALL was diagnosed in 84 cases (73.6%) using FAB criteria. Lymphoblasts of 70 cases (82.3%) showed PAS reactivity with dot like and/or discrete granular without diffuse background. 50 cases were subclassified as ALL-L1 and 20 cases as L2. Fifteen cases were not subclassified and were designated as ALL, not otherwise specified One of these was definitively AML-M0 proven by flow cytometry.

Flow cytmetry: The antigen expression of lymphoid lineages is shown in Table 3. Flow cytometric immunophenotyping identified B lineage ALL in 72 (85.7%) cases including 62 (86.1%) cases of early pre-B (CD10+) ALL, 7 (9.7%) early pre-B (CD10-) ALL and 3 (4.2%) pre B ALL (cIgM+). Expression of myeloid-associated antigens including CD11b, CD13, CD15, or CD33 was detected in 10 cases of B lineage ALL. Flow cytometry detected T lineage ALL of 12 (14.3%) cases (Table3). The most frequently expressed T cell antigens were cCD3, CD7 and CD5, followed by CD2 and CD8. The expression of myeloid associated antigens (CD11b) was observed in 2 cases.

Acute Myeloid Leukemia

Morphology and Cytochemistry: 30 (26.3%) cases were AML. The FAB M2 type was the most common (33.3%) followed by M1 (30.0%). MPO enzyme reactivity was strong in M1, M2 and M3. Six cases were M4/M5 cell type, blasts of which had moderate amounts of gray-blue cytoplasm with occasional vacuoles, cleaved nuclei and small nucleoli. Cytochemical staining for MPO was negative in only one case. Cytochemical profile (Table2)

Table 1. Characteristics of pediatric acute leukemia patients at diagnosis

Number of patients	114
Sex	
Male	75
Female	39
Age (year), median (range)	6 (0-15)
Hb (g/dL), mean (range)	8.4 (2.9-14.6)
Hct (%), mean (range)	24.9 (8.7-40.7)
WBC (x10^9/L), mean (range)	55.6 (3.9-702.9)
Platelet (x10^9/L), mean (range)	81.3 (2.6-124.6)
% of blast cell in peripheral blood, mean (range)	46.1 (0-100)
% of blast cell in bone marrow, mean (range)	79.5 (32-100)

Hb = hemoglobin; Hct = hematocrit; WBC = white blood cell

Table 2. Enzyme cytochemical staining feature of childhood acute leukemia

FAB	% of blast mean (range)	MPO	PAS	CAE	ANBE
ALL (N=85)					
L1 (N=50)	75.1 (32-100)	negative	50/50[a] 21.6[b] (15-70)	negative	negative
L2 (N=20)	85.3 (50-100)	negative	18/18 26.3 (10-75)	negative	negative
ALL morphology (N=15)	80.9 (56-98)	negative	negative	negative	negative
AML (N=29)					
M1 (N=9)	91.6 (90-96)	9/9 65.2 (11-100)	6/9 48.8 (0-100)	negative	negative
M2 (N=10)	67.7 (35-84)	10/10 86.5 (50-100)	10/10 72.4 (42-100)	10/10 54.5 (22-83)	negative
M3 (N=1)	80.0	1/1 100.00	1/1 80.00	1/1 77.00	negative
M4 (N=3)	40.0 (32-50)	3/3 50 (20-50)	3/3 50.6 (20-72)	3/3 40 (30-50)	3/3 38.3 (35-40)
M5 (N=3)	82.0 (74-90)	2/3 37.0 (0-75)	2/3 30.0 (0-60)	negative	3/3 97.7 (95-100)
M6 (N=1)	68.0	1/1 20	1/1 40	negative	negative
AML morphology (N=2)	62.0 (34-90)	negative	negative	negative	negative

a = number of positive patients/ number of tested patients; b = mean percentage of positive blast cells (range)

Table 3. Distribution of B and T lineage ALL cases according to flow cytometric immunophenotype

Markers	CD10	CD19	CD20	CD22	cCD79a	cIgM	TdT	HLA-DR	CD34	CD11b	CD13	CD15	CD33
B-cell ALL(N=72)													
Early pre B CD10+VE (N=62)	62/62/100[a]	62/62/100	22/62/35	16/55/28	50/50/100	0/50/0	35/62/56	59/62/95	46/62/74	4/62/6	1/62/2	1/62/2	6/62/10
Early pre B CD10-VE (N=7)	0/7/0	7/7/100	0/7/0	0/7/0	7/7/100	0/7/0	4/7/57	7/7/100	6/7/86	0/4/0	2/7/29	0/7/0	0/7/0
Pre B cIgM+VE (N=30)	2/3/67	3/3/100	3/3/100	3/3/100	3/3/100	3/3/100	1/3/33	3/3/100	2/3/67	0/3/0	0/3/0	0/3/0	0/3/0
Markers	**CD10**	**CD2**	**cCD3**	**CD3**	**CD4**	**CD5**	**CD7**	**CD8**	**CD34**	**TCR**	**TdT**	**HLA-DR**	**CD11b**
T-cell ALL(N=12)	2/7/29	5/5/100	7/7/100	0/7/0	1/7/14	6/7/86	7/7/100	2/7/29	4/7/57	0/7/0	5/7/71	1/7/14	1/7/14
Precursor T (N=7)													
Mature T (N=5)	0/5/0	4/5/80	ND	5/5/100	1/5/20	5/5/100	5/5/100	4/5/80	1/5/20	4/4/100	2/5/40	0/5/0	1/5/20

a = number of positive patients/ number of tested patients/ % of positive cases; N= number of patients; ND – not done

of blasts of the AML-M5 (3 cases) subtype exhibited the high proportion of blasts that were positive for ANBE in all tested patients. CAE was moderately positive in 35-40% of non-erythroid marrow cell in all cases with the AML-M4 subtype. Erythroblast of AML-M6 was reactive with granular pattern of PAS staining. Two cases of unclassified AML using enzyme cytochemical staining were designated for M0 and M7 (CD61+) by flow cytometric immunophenotype (Table 4).

Flow cytometry: The expression of CD34 (stem cell marker), CD117(c-kit) and HLA-DR were most frequently found in M0, M1 and M2. CD33 was the myeloid marker that was commonly present in all AML subtypes, followed by CD13. Flow cytometric analysis of monoblastic lineage, M4/M5, showed abundant staining for CD33 (74% to 98% of positive cells) and CD11b (68% to 98%), with less pronounced staining for CD14 (26% to 41%) and CD13 (0% to 88%). In our study, expression of lymphoid-associated antigens including CD4, CD7, CD19 or CD56 was seen in 14 (46.6%) cases, in which CD7 is the most frequent.

Discussion

Childhood acute leukemias are a heterogeneous group of disease. Due to contemporary different treatment plans, an acute leukemia must be distinguished between lymphoid and myeloid in origins. A lymphoid leukemia is further identified as precursor B-cells or T-cells. For AML, a distinction has to be made between APL and all other FAB subtypes. In most clinical situations, this can be done using morphology and enzyme cytochemistry analysis alone. There are, undoubtedly, some cases that defy correct identification based on these simple techniques and require immunophenotyping for a proper characterization.[6] These are the situations when multicolor flow cytometry plays an important role. Despite its widespread use in clinical laboratories, however, the diagnostic usefulness and limitation of flow cytometric immunophenotype have received little attention. We have combined the usefulness of a cytochemistry and a variety of markers for various childhood acute leukemia categories to a correct diagnosis and define the critical role of flow cytometry.

In this study, 70 of 84 flow cytometry-confirmed ALL patients showed PAS reactivity with dot like and/or discrete granular. This finding indicated that this PAS pattern was specific but less sensitive due to failure to identify 14 ALL cases. For immunophenotypic analysis, 72 patients were assigned to B-ALL. In this group, the early pre-B (CD10+) or common B-ALL was the most common, while the mature B-ALL phenotype was not found. To detect B lineage, CD19 and cCD79a were more sensitive antigens than CD22 and CD20. However, we found that 5 cases of AML-M2 patients co-expressed CD19. These findings suggest that cCD79a antigens detection was important in providing evidence of B ALL in cases of lymphoid antigen positive AML. For T-ALL, CD5 and CD7 were more sensitive but less specific in diagnosing T-ALL, while cCD3 and CD3 showed opposite characteristics. Nevertheless, our data suggested that cCD3 expression was useful not only confirming the T-lineage, but also in identifying immature subtypes of T ALL. For the blast cell marker, CD34 and HLA-DR were expressed on early hematopoietic stem cells and the expression level of CD34 and HLA-DR were higher especially on the most immature hematopoietic progenitors and decreased progressively with cell maturation. In our study, the expressions of these markers were higher in B-ALL than T-ALL.

There are several studies on myeloid associated antigens positive ALL (My+ALL), which appears to be more common in adult than in children ALL.[7-8] In contrast to findings in childhood ALL, where the reported frequency of My+ALL expression range 4% to 42% of cases.[9-11] In this study, the incidence of My+ALL in children was 13.8% of B-lineage ALL. Among all the myeloid antigens examined, CD33 were the most commonly expressed. In ALL, there were

Table 4. Distribution of AML cases according to flow cytometric immunophenotype

Markes	MPO*	HLA-DR	CD34	CD117	CD13	CD33	CD11b	CD14	CD15	CD61/CD41	GPA	CD4	CD7	CD19	CD56
AML (N=30)															
M0 (N=2)	0/2/0[a]	2/2/100	2/2/100	2/2/100	0/2/0	2/2/100	0/2/0	0/2/0	0/2/0	0/2/0	0/2/0	0/2/0	1/2/50	0/2/0	0/2/0
M1 (N=9)	9/9/100	9/9/100	8/9/89	9/9/100	8/9/89	9/9/100	1/9/11	0/9/0	0/9/0	ND	ND	ND	6/9/67	0/9/0	1/9/11
M2 (N=10)	10/10/100	9/10/90	9/10/90	10/10/100	10/10/100	10/10/100	5/10/50	0/10/0	7/10/70	ND	ND	ND	0/10/0	5/9/50	4/10/40
M3 (N=1)	1/1	0/1	0/1	1/1	1/1	1/1	0/1	0/1	0/1	ND	ND	ND	0/1	0/1	0/1
M4 (N=3)	3/3/100	3/3/100	3/3/100	3/3/100	3/3/100	3/3/100	3/3/100	3/3/100	3/3/100	ND	ND	2/3/67	0/3/0	0/3/0	0/3/0
M5 (N=2)	2/3/67	2/3/67	1/3/33	1/3/33	2/3/67	3/3/100	3/3/100	3/3/100	3/3/100	ND	ND	1/3/33	1/3/33	0/3/0	0/3/0
M6 (N=1)	1/1	1/1	0/1	0/1	1/1	1/1	0/1	0/1	0/1	0/1	1/1	ND	0/1	0/1	0/1
M7 (N=1)	0/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1	0/1	1/1	0/1	ND	1/1	0/1	0/1

MPO* by cytochemistry; a = number of positive patients/ number of tested patients/ % of positive cases; N= number of patients; ND = not done

several karyotypic abnormalities, some of which were associated with FAB types, immunophenotype or both. The Philadephia chromosome and 11q23 translocation were the most frequent, which also are associated with poor prognosis.[11-12] In our study, myeloid antigens were often associated with B-ALL, especially common B-ALL, while only CD11b was detected T-ALL patients.

According to FAB classification, AML was subclassified into M0, M1, M2, M3, M4, M5, M6 and M7 according to the main morphological, cytochemical and immunological features. The immunophenotype was examined for the maturation dependent myeloid antigens: CD13, CD33, CD11b, CD14, CD15, precursor markers: CD34, HLA-DR and CD117 antigens, lymphoid-associated antigens: CD7, CD4, CD19, as well as natural killer cell associated antigen: CD56. This study showed high proportion (47%) of lymphoid associated expression AML (Ly+AML). The finding suggested that cytochemical staining should be combined with the evaluation in AML. For clinical significance, Pui C-H proposed that cases of Ly+AML may response to prenisolone, vincristine and L-asparaginase after failing on protocol for myeloid leukemia.[12]

Flow cytometry by multi-staining and visualization of pathologic cells in dot-plot reflected immunophenotypic aberrancy and degree of maturation. For this purpose, the combination of MPO, PAS, CAE and ANBE were identified in relation to immunophenotypes. The cytochemical profile of blasts was in concordance with immunophenotype, particularly in more differentiated AML subtypes, M1, M2, M3, M4, M5 and M6. The discrepancies between the cytochemistry and flow cytometric immunophenotype features of blast cells were found in this study. Our study demonstrates that multicolor flow cytometry discriminated AML from ALL and recognized 3 cases (M0, M7 and M0-like ALL morphology), which could not be distinguished by cytochemistry. Moreover, MPO, PAS, CAE and ANBE were negative in 15 cases with lymphoid morphology, one of which was definitely M0 confirmed by the immunophenotype. This finding suggested that morphologically and cytochemically alone may be not successfully diagnose ALL. Multicolor flow cytometry was also useful in distinguishing B from T lineage ALL. However, neoplastic monocytic series, M4/M5 cell type, showed low percentage of positivity to CD14. Interestingly, CAE and NABE staining is notably sensitive and specific. This report suggested that NABE and CAE should be included in the diagnosis AML-M4/M5 subtypes.

In conclusion, this study demonstrated that cytochemistry together with flow cytometry immunophenotype is worthwhile using for diagnosis and subclassification of childhood acute leukemias.

References

1. *Pui C-H. Childhood leukemia. N Engl J Med 1995:332:1618-30.*
2. *Bennett JM, Catovsky D, Daniel MT, et al. Proposals revised criteria for the classification of acute myeloid leukemia: a report of the French-American-British Cooperative group. Ann Intern Med 1985;103:626-9.*
3. *Bennett JM, Catovsky D, Daniel MT, et al. Proposal for the recognition of minimally differentiated acute myeloid leukemia (AML-M0). Br J Haematol 1991;78:325-9.*
4. *Pui C-H, Frederick GB, William MC. Clinical and biological relevance of immunologic markers study in childhood acute lymphoblastic leukemia. Blood 1993;82:343-62.*
5. *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;9:1783-9.*
6. *Jennings CD, Foon KA. Recent advances in flowctyometry: application to the diagnosis of hematologic malignancy. Blood 1997;90:2863–92.*
7. *Urbano IA, Matutes E, Villamor N, et al. Clinical significance of myeloid associated antigens in acute lymphoblastic leukemia. Br J Haematol 1990;75:202-8.*
8. *Guyotat D, Campos L, Shi Z-H, et al. Myeloid surface antigen expression in adult acute lymphoblastic leukemia. Leukemia 1990;4:664-9.*
9. *Pui C-H, Behm FG, Singh B, et al. Myeloid-associated expression lack prognostic value in childhood acute lymphoblastic leukemia treated with intensive multiagent chemotherapy. Blood 1990;75:198-202.*
10. *Wiersma SR, Ortaga J, Sobel E, et al. Clinical importance of myeloid-antigen expression in acute lymphoblastic leukemia of*

childhood. N Engl J Med 1991;324:800-10.

11. *Hasan SK, Karen LC, Medeiros, et al. Acute lymphoblastic leukemia: Survey of immunophenotype, French-American-British classification, frequency of myeloid antigen expression, and karyotypic abnormalities in 210 pediatric and adult cases. Am J Clin Pathol l999;111:467-76.*
12. *Pui C-H, Ramondi SC, Head DR, et al. Characterization of childhood acute leukemia with multiple myeloid and lymphoid markers at diagnosis and at relapse. Blood 1991;78:1327-37.*

การวินิจฉัยมะเร็งเม็ดเลือดขาวชนิดเฉียบพลันในเด็กโดยใช้ Cytochemistry และ Multi-color Flow Cytometric Immunophenotype

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บทคัดย่อ : *Cytochemistry และ flow cytometry เป็นเทคนิคที่ใช้ในการวินิจฉัยโรคมะเร็งเม็ดเลือดขาวชนิดเฉียบพลันในวัยเด็ก การศึกษานี้เพื่อประเมินประโยชน์ของเทคนิคดังกล่าวในการวินิจฉัยโรคมะเร็งเม็ดเลือดขาวชนิดเฉียบพลันในวัยเด็ก โดยเป็นการศึกษาแบบย้อนหลังในผู้ป่วยรายใหม่จำนวน 114 ราย (ชาย 75 รายและหญิง 39 ราย) ค่ามัธยฐานของอายุ 6 ปี (0-15 ปี) ค่าทางโลหิตวิทยาแสดงภาวะซีด (hemoglobin<10 g/dL) และเกล็ดเลือดต่ำ (น้อยกว่า 100 x10^9/L) ร้อยละ 71 และ 80 ตามลำดับ โดย ร้อยละ 66 ของผู้ป่วยพบ blast ในกระแสเลือดมากกว่าร้อยละ 25 พบผู้ป่วยเป็น acute myeloid leukemia (AML) 30 ราย (ร้อยละ 26.3) โดยเป็นชนิด FAB M2 มากที่สุด การย้อม myeloperoxidase ให้ผลบวกแรงกับ FAB M1 ถึง M3 ผล flow cytometry พบว่า CD13 และ CD33 ให้ผลบวกกับ AML เกือบทุกราย การแสดง aberrant lymphoid antigen พบมากถึงร้อยละ 47 โดยพบ CD7 มากที่สุด พบ acute lymphoblastic leukemia (ALL) จำนวน 84 ราย (ร้อยละ 73.7) โดยพบชนิด B lineage ร้อยละ 85.7 และ T lineage ร้อยละ 14.3 ชนิดย่อย early pre B ALL (CD10+) พบมากถึง 62 ราย (ร้อยละ 86.1) และพบการแสดง aberrant myeloid antigen ร้อยละ 14 เป็นชนิด CD11b, CD13, CD15 และ CD33 การศึกษานี้แสดงถึงประโยชน์ของการใช้ cytochemistry และ flow cytometry ที่มีต่อการวินิจฉัยที่แม่นยำและสามารถแยกชนิดย่อยมะเร็งเม็ดเลือดขาวชนิดเฉียบพลันในวัยเด็ก*

Key Words : ● *มะเร็งเม็ดเลือดขาวชนิดเฉียบพลัน* ● *cytochemistry* ● *flow cytometry*

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต 2554;21:23-31.