STUDY ON THE IMMUNOPHENOTYPING OF ACUTE LEUKEMIA IN CHIDREN TREATED AT HUE CENTRAL HOSPITAL

Tran Thi Phuong Tuy[1], Nguyen Duy Thang[1], Nguyen Thi Hong Hanh[1], Dong Si Sang[1], Phan Thi Thuy Hoa[1], Ngo Tu Cuong[1], Phan Hoang Duy[1], Le Thi Phuong Lan[1], Nguyen Thi Thu Hien[1], Chau Van Ha[1], Ton Nu Tra Mai[1], Nguyen Thi Bich Tuyet[1]

ABSTRACT

***Objective**: to study on the immunophenotyping of acute leukemia in chidren treated at Hue Central Hospital*

***Methods**: 41 newly diagnosed acute leukemia children were hospitalized in Hue Central Hospital from January 2013 to December 2013 by using cross sectional study and using the FAB classification and completing by the 1995 EGIL and the 2008 WHO immunophenotyping classification.*

***Results**: Male/Female: 2.4/1. Age ranged to 1 month from 15 years (mean: 6.6 ± 4.9). By using the FAB classification and completing by EGIL (1995) and WHO (2008) immunophenotyping classification for 41 patients, the results showed that: the ratio of AML was 41.5% (17 cases), the ratio of B-ALL: 53.7% (22 cases), the ratio of T-ALL: 4.8% (2 cases). The group of the aberrant antigen AL was seen in 11 cases (26.8%) (included ▪ Lym T+ AML: 2 cases; Lym B+AML: 2 cases; the most aberrant antigen was CD19+: 50%, the next was CD7+: 25% and the CD2+: 25%; ▪ Myeloid+ B-ALL: 6 cases, the most aberrant antigen was: CD13+: 50%, the next was CD33+: 33.3% and the last was CD15+: 17.7%; ▪ LymB+ T-ALL: 1 case: the aberrant antigen was CD20+. In AML: CD34+: 70.6%, HLA DR: 80%; in B-ALL: CD34+: 76.2%, HLA-DR+: 100%.*

***Conclusion**: These results contributed effectively to improve the classification and the proper diagnosis and treatment for children with acute leukemia at Hue Central Hospital.*

***Key words**: Acute Leukemia (AL), Acute Myeloid Leukemia (AML), Acute Lymphoid Leukemia (ALL), immunophenotyping classification, Cluster of Differentiation (CD), aberrant antigens, mixed phenotype acute leukemia (MPAL).*

I. INTRODUCTION

Leukemia is one of the most common neoplasms in children. It includes acute leukemia and chronic leukemia. Acute leukemia is classified such as acute lymphoid leukemia and acute myeloid leukemia, characterized by different clinical and cytological features [7]. The accurate diagnosis of the type of acute leukemia to contribute effectively to improve the classification and the proper diagnose and treatment for children with acute leukemia at Hue Central Hospital.

Objective: to study on the immunophenotyping

1. Hue Central Hospital

Corresponding author: Tran Thi Phuong Tuy
Email: phuongtuy07@gmail.com
Received: 26/10/2014; **Revised**: 17/11/2014 by Nguyen Van Tranh
Accepted: 5/12/2014

of acute leukemia in chidren treated at Hue Central Hospital.

II. MATERIALS AND METHODS

2.1. Patients: From January 2013 to December 2013, 41 newly diagnosed acute leukemia children (aged < 15 years) were hospitalized in Hue Central Hospital.

2.1.1. Inclusion criteria:

- Clinical: infectious syndrome, anemia, hemorrhage, splenomegaly, hepatomegaly, bone pain.

- Laboratory evaluation:

* Hemogramme: red blood count, leukocyte count, platelet count, % blast cell.

* Myelogramme: Morphology diagnosis was based on the FAB criteria, the diagnosis of AL is confirmed when ≥ 30% blasts are present in the bone marrow.

* Cytochemistry: bone marrow smears were stained using May Grunwald - Giemsa, Periodic Acid - Schiff, Sudan Black, Myeloperoxidase and Nonspecific

Esterase and Nonspecific Esterase inhibited by NaF (some cases).

* Immunophenotyping: the panel of monoclonal antibodies was CD2, CD3, CD5, CD7 for T lineage ALL, CD19, CD20, CD22 for B lineage ALL, CD13, CD14, CD15, CD33, for myelomonocytic lineage AL, CD41a for megakaryocytic AL, anti glycophorin A for erythrocytic AL, CD34, CD10 and anti HLA-DR for non lineage.

2.1.2. Exclusion criteria: prior treatment, secondary AL to the other diseases.

2.2. Methods:

2.2.1. Cross sectional study.

2.2.2. Tests:

- Count blood cell in CellDyn 3200 machine

- Bone marrow aspiration

- Stain the blood and bone marrow smears by Giemsa method

- Bone marrow smears were stained using Periodic Acid - Schiff, Sudan Black, Myeloperoxidase and Nonspecific Esterase and Nonspecific Esterase inhibited by NaF (some cases).

- Using the FAB classification and completing by the 1995 EGIL and the 2008 WHO immunophenotyping classification by the panel of monoclonal antibodies fixed with FITC or PE on 2 colours Becton Dickinson was CD34(8G12) PE, CD33PE, CD13PE, CD14PE, CD15FITC, CD2PE, BD Simultest CD3 FITC/19PE, CD5PE, CD7FITC, CD10 anti -CALLA, CD20PE, CD22PE, CD41aFITC, GlycophorinA.

- Immunofluorescence studies were evaluated by fluorescence microscopy, using a Olympus fluorescence microscope. When 20% or more of the cells were positive for a particular marker, the case was considered to be positive for that marker. An exception was made for anti -MPO, CD3 due to their high degree of specificity, being cut-off point was 10% of blast cells stained. These cut-off points are applicable to both diagnosis of the acute leukemias and classification of the various ALL and AML subtypes [3].

2.2.3. Criteria for the immunological classification of acute leukemias was proposed by EGIL on 1995 [3]:

- Classification of ALL:

* B - ALL : CD 19+ and/or CD22+, CD 10+/-, CD 20+/-, cytoplasmic IgM+, cytoplasmic/ surface kappa or lambda+

* T - ALL : cytoplasmic/ membrane CD3+, CD7+, CD2+ and/or CD5+, and/or CD8+, CD1a+, anti TCR α/β +, anti TCR γ/δ +.

* ALL with myeloid antigen expression (My + ALL)

- Classification of AML:

* Myelomonocytic lineage (possitive for at least 2 or more myeloid markers: anti MPO+, CD13+, CD33+, CDw65+ and/or CD117+

* Erythroid lineage (pure erythroid, M6):

Early/immature: unclassifiable by markers

Late/mature: anti glycophorin A+

* Megakaryocytic lineage (M7): CD 41+ and/or CD61 + (cytoplasmic/ membrane)

* Early myeloid (M0): (only determine by immunological markers): phenotype as other myelomonocytic AML but negative cytochemistry and lymphoid specific markers: CD3, CD79a, CD22.

* TdT+ AML

* AML with lymphoid antigen expression (Ly + AML).

- *Undifferentiated acute leukemias*: the blast cells do not express lineage specific markers. These cases are often CD34+, class II HLA- DR +, CD38+ and CD7+. At present, the nature of the blasts can not be clarified by immunophenotyping requiring other studies to demonstrate the early myeloid or lymphoid commitment of the leukemic cells.

- *Bilineal acute leukemia and biphenotypic acute leukemia* are now collectively considered as "mixed phenotype acute leukemia" (MPAL)(according to the 2008 revision of WHO classification of myeloid neoplasms and acute leukemia) [10]:

* Myeloid lineage:

Myeloperoxidase (flow cytometry, immunohistochemistry, or cytochemistry) or Monocytic differentiation (at least 2 of the following: nonspecific esterase, CD11c, CD14, CD64, lysozyme)

* T lineage:

Cytoplasmic CD3 (flow cytometry with antibodies to CD3 epsilon chain; immunohistochemistry using polyclonal anti CD3 antibody may detect CD3 zeta chain, which is not T cell-specific) or Surface CD3 (rare in MPAL).

* B lineage:

Strong CD19 with at least 1 of the following strongly expressed: CD79a, cytoplasmic CD 22, CD10 or Weak CD19 with at least 2 of the following strongly expressed: CD79a, cytoplasmic CD22, CD10.

2.3. Statistical analysis: Statistical analysis was performed by SPSS 11.5 software.

III. RESULTS

From January 2013 to December 2013, 41 new acute leukemia children were diagnosed and classificated by FAB criteria, completing by immunophenotyping classification by EGIL (1995) and WHO (2008), there were the results:

3.1. General features

Age: 1 month to age of 15, mean: 6.6 ± 4.9.

Gender: male: 29 cases (70.7%), female: 12 cases (29.3%), male/female: 2.4/1.

3.2. Features of immunophenotyping in acute leukemia

Table 1: The parameters of the blood and bone marrow at the time of diagnosis:

Parameters	Min	Max	Mean ± SD
Hb (g/l)	49	125	83.90 ± 15.08
WBC (x10^9/l)	1.7	489	73.04 ± 15.08
PN (x10^9/l)	0.15	49.05	5.65 ± 9.27
Plt (x10^9/l)	7	381	71.70 ± 82.25
Blast/ blood (%)	0	87	34.24 ± 26.09
Blast/ bone marrow (%)	30	93	59.27 ± 16.12

Table 2: The immunophenotyping classification of AL cases:

Types	n	%
AML	13	31.7
ALL	17	41.5
MPAL	11	26.8
Total	41	100.0

Total: AML: 17 cas (41.5%); ALL: 24 cas (58.5%).

Table 3: The immunophenotyping classification of subtypes of AL cases:

Subtypes	n	%
M0	1	2.4
M1	5	12.2
M2	4	9.8
M3	1	2.4
M4	3	7.3
M5	3	7.3
M6	0	0
M7	0	0
B - ALL	22	53.7
T - ALL	2	4.8
Total	41	100.0

Table 4: The ratio of the aberrant antigens in the MPAL groups

Aberrant antigens		AML* (n=4) (23.5%)		B - ALL* (n=6) (27.3%)		T - ALL* (n = 1) (50%)
		n	%	n	%	n
T Lineage	CD 5+	1	25.0	0	0	-
	CD7+	1	25.0	0	0	-
B Lineage	CD19+	2	50.0	-	-	0
	CD20+	0	0	-	-	1
Myeloid Lineage	CD13+	-	-	3	50.0	0
	CD15+	-	-	1	17.7	0
	CD33+	-	-	2	33.3	0

(*): The AL had the aberrant antigens.

(-): The antigens of their own AL groups (not display data).

Comment: CD19+ was the most common CD in the AML*; CD13+ was the most common CD in the B-ALL*

Table 5: The ratio of the CD+ in the AL groups

CD +	AL (n = 41)		AML (included AML*) (n = 17)		ALL (included ALL*) (n = 24)	
	Cases (+)/ cases tested	%	Cases (+)/ cases tested	%	Cases (+)/ cases tested	%
CD 34 +	28/40	70.0	12/17	70.6	16/23	69.6
HLA DR+	18/21	85.7	8/10	80.0	10/11	90.9
CD 10+	11/41	26.8	0/17	0	11/24	45.8
CD 33+	17/39	43.6	15/16	93.8	2/23	8.7
CD 13+	19/39	48.7	17/17	100.0	2/22	9.1
CD 14+	7/38	18.4	7/17	41.2	0/21	0
CD 15+	18/39	46.1	17/17	100.0	1/21	4.8
CD 2+	2/38	5.3	0/16	0	2/22	9.1
CD 3+	1/38	2.6	1/23	4.3	0/15	0
CD 5+	3/39	5.1	1/16	6.3	2/23	8.7
CD 7+	3/39	5.1	1/17	5.9	2/22	9.1
CD 19+	25/40	62.5	3/16	18.8	22/24	91.7
CD 20+	12/28	42.9	0/13	0	12/15	80.0
CD 22+	19/39	48.7	0/16	0	19/23	82.6

Table 6: The incidences of the CD34+, CD10+, HLA-DR+ in the AL cases

	AML (n=17) Cases (+)/cases tested (%)	B-ALL (n=22) Cases (+)/cases tested (%)	T-ALL (n=2) Cases (+)/cases tested (%)
CD 34+	12/17 (70.6)	16/21 (76.2)	0/2
HLA DR+	8/10 (80)	9/9 (100)	1/2
CD 10+	0/17 (0)	11/22 (50)	0/2

IV. DISCUSSION

4.1. General features

Age: The youngest: 1 month, the eldest: 15 years, mean of age: 6.6 ± 4.9.

This approximated to the study of Mirbehbahani: the youngest: 6 month, the eldest: 15 years, mean of age: 5.96 ± 3.92 (n = 62) [5].

Gender: male/female: 2.4/1, this ratio approximated to the study of Mukda: 1.9/1; The study of Nguyen Cong Khanh and of Mirbehbahani had the lower rate: 1.68/1 and 1.21/1 respectively [1], [5], [6].

4.2. Features of immunophenotyping in acute leukemia

We compare between results of our study and Mukda study.

Table 6: The characteristics of AL children at the time of diagnosis

Parameters	Mukda (2010)	Our study
n	114	41
Male/female	1.9/1	2.4/1
Mean of age (min - max)	6 (0 - 15)	6.6 (0 - 15)
Hb (g/l) (min - max)	84(29 - 146)	83.9 (49 - 125)
WBC ($x10^9$/l) (min - max)	55.6 (3.9 - 702.9)	73.0 (1.7 - 489)
PN ($x10^9$/l) (min - max)		5.7(0.15 – 49.05)
PltC ($x10^9$/l) (min - max)	81.3 (2.6 - 124.6)	71.7 (7 - 381)
Blast/blood(%) (min - max)	46.1 (1 - 100)	34.2 (0 - 87)
Blast/bone marrow (%) (min - max)	79.5 (32 - 100)	59.3 (30 - 93)

Our results approximated to that of Mukda study [6].

The immunophenotyping classification of AL cases: This study: AML were 31.7%; ALL were 41.5% and MPAL were 26.8%; to be constrast with Nguyen Cong Khanh et al: AML were 31%, ALL were 62% and MPAL: 7.0% (n = 142) or Shen HQ et al: AML were 35.1%, ALL were 55.9% and MPAL were 8.1% (n = 222), this difference could be due to the small number of cases in our study [1], [8].

The immunophenotyping classification of subtypes of AL cases: This study was found only one case M0 AML (2.4%) and one case M3-AML (2.4%). Immunophenotyping at diagnosis is specially precious for the identification of morphologically undifferentiated M0 AML cases, accurate diagnosis of the hypogranular M3 variants [4]. Although some AMLs, eg M3 AML are, unlike other AML subtypes, usually HLA-DR-, CD34-, this phenotype is not consistent in M3-AML and not specific enough as it may be found in other AML, eg late M2-AML [3]. In this study, there were neither M6 -AML nor M7- AML. For Bene M.C., the need for an early marker specific for erythoid lineage was

discussed as it is likely that cases resulting from the proliferation of primitive erythroid cells are underdiagnosed [3].

The incidence of the aberrant antigens in MPAL cases: The incidence of the aberrant antigens in ALL cases of this study was 29.2 (7/24 cas), approximated Supriyadi E.: 25%, for Naeim F. there were about 10-30% ALLs may express myeloid-associated antigens such as CD13 and CD33. For Supriyadi E. the variation of those findings may be due to variations in definition, the number of monoclonal antibodies used. Besides these more technical explanations, the myeloid antigen expression may also differ due to ethnical differences [7] [9]. In B-ALL, the myeloid-associated antigens CD13 was the most incidence: 50%, then: CD33: 33.3% and the lowest was CD15: 17.7%, in study of Shen HQ was CD13: 18.5%, then CD15: 11.3%, and the lowest was CD33: 4.3% [8]. For Naeim F. CD33, CD13 and CD11b appear to be the most commonly expressed myeloid - associated antigens in ALLs. CD15 is frequently expressed in ALLs with t (4;11) [7].

In this study, for T-ALL cases, there were 2 cases, in which one cases expressed aberrant antigen CD20. For Naeim F. lymphoid-associated antigens such as CD10, CD20 and CD22 to be expressed on blast cells of approximately 10-15% of AML [7].

In AML cases, there were 2 cases expressed CD19+: 4.8% (2/41 cases), approximated to Mukda E. in which 5 AML cases expressed CD19+: 4.4% (5/114 cases). For Mukda E., to detect B lineage, CD19 and cCD79a were more sensitive antigens than CD22 and CD20 (this study was in agreement with: CD 19+: 100%, CD22+: 90.5% và CD20+: 84.6%) [4] [6]. The new WHO classification uses CD19, together with at least one antigen among CD10, cCD22 and CD79a to define B-cell lineage. However, CD19 expression is seen approximately in one third of AML with t (8;21)(q22;q22) and serves the predict the presence of this cytogenetic abnormality in AML. Thus, if CD19 and CD79a are employed as B-cell lineage-specific markers, some AML will be diagnosed as biphenotypes AL [7], [11].

2 cases AML expressed the aberrant antigens CD5+ and CD7+ had the same incidence: 2.4% (1/41 cases), for Zhao CD2, CD5 and CD7 were commonly found in AML; for Shen HQ the most commonly aberrant antigen included CD7 (12.8%), then CD19 (6.4%) and the last: CD2 (5.1%) [8], [11]. For Naeim F. expression of lymphoid-associated antigens has been reported in up to 60% of chilhood AMLs and CD7 is expressed in a large propotion of AMLs [7].

The ratio of the CD+ in the AL groups: In this study, the CD19+, CD10+ và HLA-DR+ in the ALL group was found in high expressed antigens: 91.7%, 45.8% and 90.9% respectively, approximately with the study of Mirbehbahani were: CD19+(90.2%), CD10+(84.36%) HLADR+(70.58%) [5]. It should be noted that there are variety in these antigens in ALL patients of different regions [5]. It was outlined that CD10 may be positive in some T-ALL and AML, but unlike in B-ALL, this marker is not taken into account for the classification in the various subtypes [3], [11].

For the blast cell marker, CD34 and HLA-DR were expressed on early hematopoietic stem cells and the expression level of CD34 và HLA-DR were higher especially on the most immature hemopoietic progenitors and decreased progressively with cell maturation. In the study of Mukda, the expression of these marker were higher in B-ALL than T-ALL like our study [6].

V. CONCLUSION

41 newly diagnosed acute leukemia children included male/female: 2.4/1. The age ranged to 1 month from 15 years (mean: 6.6 $\pm$ 4.9). By using the FAB classification and completing by EGIL (1995) and WHO (2008) immunophenotyping classification, the results showed that: the ratio of

AML was 41.5% (17 cases), the ratio of B-ALL: 53.7% (22 cases), the ratio of T-ALL: 4.8% (2 cases). The group of the aberrant antigen AL was seen in 11 cases (26.8%) (included ▪ Lym T+ AML: 2 cases; ▪ Lym B+ AML: 2 cases; the most aberrant antigen was CD19+: 50%, the next was CD7+: 25% and the CD2+: 25%; ▪ Myeloid+ B-ALL: 6 cases, the most aberrant antigen was: CD13+: 50%, the next was CD33+: 33.3% and the last was CD15+: 17.7%; ▪ LymB+ T-ALL: 1 case: the aberrant antigen was CD20+. In AML: CD34+: 70.6%, HLA-DR: 80%; in B-ALL: CD34+: 76.2%, HLA-DR+: 100%.

REFERENCES

1. Nguyen Cong Khanh (2006), Studying on the classification of childhood acute leukemias at the Central Pediatric Hospital, *Journal of Practical Medicine*, 545, pp. 118 - 122.
2. Nguyen Ngoc Minh (2007), *Cluster of Differenciation,* Lessons of Post-graducate for Haemology and Blood Transfusion, Ha Noi, pp 64 -103.
3. Bene M.C. (1995), Proposals for the immunological classification of acute leukemias, (EGIL), *Leukemia*, 9, pp. 1783 - 1786.
4. Bene M.C. (1999), Impact of immunophenotyping on management of acute leukemia, Haematologica, 84, pp. 1024 - 1034.
5. Mirbehbahani NB (2011), Immunophenotyping of leukemia in children, Gorgan, Iran, *Iranian Journal of Pediatric Hematology Oncology*, 1(4), pp. 115 - 120.
6. Mukda E. (2010), Cytochemistry and Multi-color flow cytometric Immunophenotype for diagnosis of childhood acute leukemia, *J Hematol Transfus Med,* 21, pp. 23- 31.
7. Naeim F. (1999), *Pathology of bone marrow,* Williams & Wilkins, pp. 220 - 240.
8. Shen HQ (2003), Immunophenotyping of 222 children with acute leukemia by multi - color flow cytometry, *Zhonghua er ke za Zhi, Chinese Journal of Pediatrics,* 41(5), pp. 334 - 337.
9. Supriyadi E. (2012), Myeloid antigen expression in childhood acute lymphoblastic leukemia and its relevance for clinical outcome in Indonesian ALL- 2006 Protocol, *Journal of Oncology*, 2012, pp. 1 - 7.
10. Vardiman J.W. (2009), The revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes, *Blood,* 114(5), pp. 937- 951.
11. Zhao X. F. (2010), Diagnosis of biphenotypic acute leukemia: a paradigmatic approach, *Int J Clin Exp Pathol*, 3(1), pp. 75- 86.