THE VALUE OF MORPHOLOGY AND CYTOCHEMISTRY VIA IMMUNOPHENOTYPING IN THE DIAGNOSIS OF CHILDHOOD ACUTE LEUKEMIA

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ABSTRACT

Acute leukemia is the most commont cancer in children and is curable if correctly diagnosed and effectively treated. The diagnosis and accurate cloning of acute leukemia play an important role in the treatment resμlts for regimens and prognosis between two myeloid and lymphoid completely different. A descriptive cross- sectional study was carried out to compare the value of morphology and cytochemistry via immunophenotyping in the diagnosis of childhood acute leukemia. Research conducted from April 2017 to Jμly 2019 received 63 cases of patients diagnosed and treated at Pediatrics Center in Hue Central Hospital. The morphology was effective of AML and ALL were 92.1% and 95.2% respectively. The value of cytochemistry with Myeloperoxydase, Sudan black and P.A.S were 86% and 88%, 88.9% and 92.6%, 73.3% and 73.3% respectively. In current condition of developing countries, an effort to standardize morphology and cytochemistry woμld be cost – effective to immunophenotype for classification and differentiation of acute leukemia.

Key words: *childhood acute leukemia, morphology, cytochemistry, immunophenotyping.*

I. INTRODUCTION

Acute Leukemia (AL) is one of the most common type of cancer in children, nearly 30% of childhood cancers, in which acute lymphocyte leukemia (ALL) is 5 times greater than acute myeloid leukemia (AML) [6]. In the 1960s, there wasn't chemotherapy; the survival rate of leukemia patients is very low. But nowadays, by using multiple chemotherapy regimens, the prognosis has improved a lot, especially with ALL. According to a study by the Child Cancer Group published in 2012, the after-5-year survival rate in the 2000-2005 period reached 90.4% [4].

The diagnosis, the correct classification is very important in treatment result because the therapy and prognosis between the two cell lines are completely different. Previously, the classification was based mainly on cell morphology and cytochemistry. Both of these methods are easy to implement, simple, inexpensive, and have quick results but depend very much on subjective factors so the reliability and specificity are not high. The inspection of immunophenotype by flow cytometry has established a technique to examine cellular immunological imprints to identify abnormal cell populations in blood or bone marrow, therefore determine the nature of cell lines, level of differentiation of the cells and be able to identify in cases that cells are transformed, malformed, undifferentiated or poorly differentiated, or carrying imprint of two lines at the same time. However, this method is expensive and requires

modern equipment that may not be suitable in developing countries [5].

In the actual situation of our country, it is necessary to evaluate and find the suitable and effective AL diagnostic methods that can be widely applied in many health facilities. Therefore, to evaluate the applicability of two simple methods, cell morphology and cytochemistry, in the diagnosis of AL disease, we carried out the study "the value of morphology and cytochemistry via immunophenotyping in the diagnosis of childhood acute leukemia" with the following objectives:

1. Describing clinical and subclinical characteristics of childhood acute leukemia

2. Comparing the values of cell morphology - cytochemistry to immunophenotyping in the diagnosis of childhood acute leukemia.

II. SUBJECTS AND METHODS

2.1. Subject: Including 63 childhood acute leukemia patients who were diagnosed and treated with AL at Pediatric Center, Hue Central Hospital of Vietnam from April 2017 to July 2019.

2.2. Methods

2.2.1. Study design: Descriptive cross-sectional study

2.2.2. Study method:

Collecting clinical and subclinical data, performing bone marrow tests to assess cell morphology, cytochemistry with Myeloperoxydase, Sudanese black, P.A.S. Comparing to the results of immunophenotyping analysis on BD FACSCanto machine with different cluster antigens for myeloidcell, B lymphocyte, T lymphocyte, non-specific markers and others [5].

Table 2.1. Cytochemistry characteristics of some blood cell lines

	Neutrocytes	Monocytes	Red blood cells	Lymphocytes
Myeloperoxydase	+++	± → ++	-	-
Sudan black	+++	+ → ++	-	-
P.A.S	±	±	±	- → +++

Thereby calculating the sensitivity, specificity, positive predictive value, negative predictive value and diagnostic value of the cell morphological and cytochemical methods compared to the immunophenotyping method which is called "the gold standard" in AL diagnosis.

III. RESULTS

There were 63 eligible AL patients for this study. The average age was 4.2 years (minimum 29 days). 58.7% are boys. There were 45 ALL patients, accounting for 71.4%, 16 AML patients, accounting for 25.4%; especially there were 2 patients with an immunological imprints of both myeloid and lymphocyte populations, classified as biphenotype.

Table 3.1. Clinical characteristics

	n	%
Fever	29	46,0
Hemorrhage	23	36,5
Amenia	54	85,7
Hepatomegaly	33	52,4
Splenomegaly	27	42,9
Lymphadenopathy	28	44,4
Osteoarthritis pain	10	15,9

Anemia was the most common symptom in pediatric AL patients (85.7% of the total cases). Other symptoms such as fever, hemorrhage, hepato-splenomegaly, lypmphadenopathy were also common (> 40%). Especially, 15.9% of children showed osteoarthritis pain, in some cases it was the only symptom at the hospitalization, making the initial diagnosis mistaken for osteomyelitis, systemic juvenile arthritis.

Table 3.2. Characteristics of blood count changes

		n	%	Average (min – max)
White blood cells	< 10k/µl	27	42.9	59.4 (1.0 -609.5)
	10 – 50	21	33.3	
	> 50	15	23.8	
Hemoglobin	< 6g/dl	17	27.0	7.6 (2.7 – 12.3)
	6 – 12g/dl	44	69.8	
	> 12g/dl	2	3.2	
Platelets	< 20k/µl	12	19.0	75.5 (4 – 420)
	20 – 50k/µl	20	31,7	
	> 50k/µl	31	49.3	
Blast	< 25%	42	66.7	19.1 (0 – 94)
	25 – 50%	14	22.2	
	> 50%	7	11.1	

Most children with AL had not leukocytes (42.9%), 23.8% of children had elevated white blood cell count > 50k/µl, the average number of leukocytes was 59.4k/µl, the case with the highest white blood cell count reached 609.5k/µl.

More than 95% of children had anemia, of which the average hemoglobin level is 7.6g/dl, one patient was admitted to the hospital with the lowest hemoglobin level of only 2.7g/dl.

The average platelet count was 75.5k/µl. Platelet counts ranged from 4 - 420k/µl.

Table 3.3. Result of classification according to cell morphology – cytochemistry and immunophenotyping

ALL		Immunophenotyping		
		AML	*Biphenotype*	
Cell morphology (N = 63)	*ALL*	44	2	2
	AML	1	14	0
Myeloperoxydase (N = 50)	*Positive*	0	8	0
	Negative	35	6	1
Sudan đen (N = 27)	*Positive*	0	6	0
	Negative	18	2	1
P.A.S (N = 15)	*Positive*	6	2	0
	Negative	2	5	0

All patients were tested by using both methods cellular morphology, and cellular immunology, comparing the results of these two methods shows a high degree of similarly, 5 patients showed different results, of which there were two Biphenotype cases, that were ALL and according to bone marrow analysis.

50 children took Myeloperoxydase tests, 27 children took Sudan black test, only 15 took PAS test, the results also showed a high similarity when compared to immunophenotyping. One biphenotype case gave negative results to both Sudan black and Myeloperoxydase.

Table 3.4. Cellular morphology – cytochemistry diagnosis value in cellular lines classification

		Sensitivity	Specificity	Positive predictive value	Negative predictive value	Diagnosis value
Cell morphology	*ALL*	97,8%	77,8%	91,7%	93,3%	92,1%
	AML	87,5%	97,9%	93,3%	95,8%	95,2%
Cytochemistry						
Myeloperoxydase	*ALL*	100,0%	53,3%	83,3%	100,0%	86,0%
	AML	57,1%	100,0%	100,0%	85,7%	88,0%
Sudan black	*ALL*	100,0%	66,7%	85,7%	100,0%	88,9%
	AML	75,0%	100,0%	100,0%	90,5%	92,6%
P.A.S	*ALL*	75,0%	71,4%	75,0%	71,4%	73,3%
	AML	71,4%	75,0%	71,4%	75,0%	73,3%

Cellular morphology had high diagnostic value in AL cellular lines classification, with more than 90% sensitivity, specificity, positive predictive value, negative predictive value. Cellular morphology specificity in diagnosis of ALL was only 77.8% due to confusion with biphenotype.

Cytochemistry with Myeloperoxydase and Sudan black had 100% ALL sensitivity, negative predictive value and 100% AML specificity, positive predictive value, Myeloperoxydase and Sudan black tests diagnostic values were quite high, nearly 90%.

Cytochemistry with P.S.A gave results with diagnostic value only in 70 – 75% range.

IV. DISCUSSION

Hue Central Hospital is the leading general medical center of the Central - Highlands region of Vietnam, in which the Pediatric Center is currently the only unit in the region receiving childhood AL patients. During the 27-month study period, there were 63 children diagnosed with AL, that showed the high morbidity of the hematopoietic system malignant diseases in children.

The average age of children is 4.2 years, Boys is more common than girls, and most are ALL accounting for 70%. These results are quite consistent with the epidemiological study in the US in 2014 with the highest diagnosis age between 2 and 4 year old, boys are more common than girls, and the main type is ALL [6].

Childhood AL patients were hospitalized with many different symptoms including fever, hemorrhage, amenia, hepatomegaly, splenomegaly, lymphadenopathy and osteoarthritis. According to a pooled analysis of Clarke and colleagues, hepatomegaly accounted for 64%, splenomegaly accounted for 61%, lymphadenopathy accounted for 41%, pale skin accounted for 54%, fever accounted for 53%, bleeding accounted for 53%, and 43% case of pain [2]. Having clinical manifestations helps physicians in early suspicion, considering the symptoms together but not separately, then making early diagnosis of childhood AL. Osteoarthritis pain occurred in 10 cases, in which some patients were misdiagnosed as osteomyelitis or systemic juvenile arthritis. For these cases, when there were unexplained symptoms, a blood test should have been performed to diagnose the disease earlier.

The mean value of blood cells count in this study: leukocytes count was 59.4k/μl, hemoglobin level was 7.6g/dl, the platelet count was 75.5k/μl. Hematologic al changes in childhood AL patients are quite different, there are some who do not have any changes in hematology, or just showing transformation of cellular line, making it difficult to diagnose.

All patients were tested by using both methods cellular morphology, and cellular immunology. Due to the lack of chemical compounds, there were 50 children who took Myeloperoxydase tests, 27 children took Sudan black test, and only 15 took PAS

test. There were two bi-phenotype cases according to cellular immunology, both had the results of cell morphology and cellular cytochemistry as ALL, one case of suspicion on cell morphology, one case of suspicion on cellular cytochemistry.

Cellular morphological method by microscopic examination of bone marrow to evaluate cell shape and FAB classification in this study showed high diagnostic value in AL classification with more than 90% sensitivity, specificity, positive predictive value, negative predictive value. This result shows that cellular morphology still has an important role in AL classification, helping to make earlier diagnosis and earlier treatment for patients.

Cytochemistry with Myeloperoxydase, Sudan black and P.A.S had high diagnostic values: 86%, 88.9% and 73.3% respectively with ALL; 88%, 92.6% and 73.3% respectively with AML. In the study of Glaucia and colleagues, diagnostic values of Myeloperoxydase, Sudan Black and P.A.S were 91%, 90.9% and 96.9% respectively [3]. In the study of Akram, the diagnostic values of these above methods were 93.33%, 93.33% and 50% respectively [1]. Cytochemistry with Myeloperoxydase and Sudan black has 100% ALL negative predictive value, so there would be 100% AML positive predictive. But for the myeloid types, these two methods can still be negative in cases of malignancy of erythrocytes or monocytes. So we could not confirm the diagnosis of ALL when the results of Myeloperoxydase and Sudan black tests were negative. As for P.A.S, the results were variable for the myeloid types which could give positive or negative results. With ALL, the results may be negative in some cases of T lymphocyte AL. But P.A.S is still valid when combined with other tests to classify types of AL.

Three Biphenotype cases in Glaucia's study all showed negative cytochemistry result [3], which is quite similar to this study results.

V. CONCLUSION

In this study, cellular morphology and cytochemistry methods have been shown to have high sensitivity and specificity in acute leukemia diagnosis at low cost when used alone or in combination. It is easy to implement, does not require motern expensive equipments, and cellular immunophenotyping gives supportive diagnosis information.

We believe that cellular morphology and cytochemistry will continue to play an important role in the diagnosis of acute leukemia, helping to predict and select initial treatment in Vietnamese health practice, in areas where there isn't modern diagnostic equipments such as immunophenotyping, cytogenetics and molecular biology yet.

REFERENCES

1. Akram A. M. D., Amal R. M., Bassma A. A. A. E. E. (2016), "The value of cytochemical stains in the diagnosis of acute leukemia". *International Journal For Research In Health Sciences And Nursing,* 2 (5), p.1-7.

2. Clarke R. T., Bruel A. V., Bankhead C. et al (2016), "Clinical presentation of childhood leukaemia: a systematic review and meta-analysis". *British Medical Journal,* 101, pp.894-901.

3. Glaucia Aparecida DR, Miriane da Costa G, Moraes-Souza H et al (2017), "The Role of Cytochemistry in the Diagnosis of Acute Leukemias". *International Journal of Health Sciences & Research,* 7 (8), pp.290-295.

4. Hunger S. P., Lu X., Devidas M. et al (2012), "Improved Survival for Children and Adolescents With Acute Lymphoblastic Leukemia Between 1990 and 2005: A Report From the Children's Oncology Group". *Journal of Clinical Oncology,* 30 (14), pp.1663-1669.

5. Smock K. J. (2019), "Examination of the Blood and Bone Marrow", *Wintrobe's Clinical Hematology 14th Edition,* Lippincott williams & wilkins, pp.182-230.

6. Ward E., DeSantis C., Robbins A. et al (2014), "Childhood and adolescent cancer statistics, 2014". *A Cancer Journal for Clinicians,* 64 (2), pp.83.