Correlation of cyto-morphology with flow cytometric immunophenotyping in acute leukemia's: A Comparative study.

Ahmed Kaid Salem[1*], Akram Yahia Al-hag[1], Nouraldin Aljaber[1], WAssem Al-Kadasy[2], Amin Abdulrab[2], Saleh Abu Hadi[3], Ali Ahmed Al-Zaazaai[3]

[1]Department of Internal Medicine, Sana'a University, Sana'a, Yemen

[2]Department of Internal Medicine, Al-Thawra Teaching Hospital, Sana'a, Yemen

[3]Department of Pharmacy, Wenzhou Medical University, Wenzhou, China

Abstract

Background: Immunophenotypic characterization of Acute Leukemia's (AL) is an important clinical application of flow cytometry (FCA) and has become a powerful tool contributing to the diagnosis and classification of acute leukemia's.

Objective: To compare the morphological and cyto-chemical diagnosis of peripheral blood and bone marrow sample with flow cytometric immunophenotypic diagnosis in acute leukemia's.

Keywords: Leukemia, Cytomorphology, Flow cytometry, AML, B-ALL.

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Introduction

Acute leukemias are a heterogeneous group of malignancies with varying clinical, morphologic, immunophenotypic, genetic and molecular characteristics. Despite the increasing importance of molecular and genetic features in the classification of acute leukemias, morphologic and immunophenotypic analysis remains the main modality to diagnose acute leukemia for initial evaluation and providing a rapid assessment to direct specific molecular genetic tests [1,2].

Acute leukemia is a medical emergency and thus requires a critical workup process for diagnosis and prompt management. Delay in the diagnosis and treatment of acute leukemia could be fatal. Acute leukemia poses a number of diagnostic and treatment challenges. Patients' symptoms may be nonspecific in nature (e.g. fatigue, weight loss) and the complete blood count will often show one or more cytopenia [3]. Many patients with undiagnosed acute leukemia will first present with life-threatening complications, including febrile neutropenia, severe anemia, and major bleeding as result of normal hematopoietic cells in the bone marrow being replaced by leukemic blasts [4].

France-American-British classification (FAB) group, based on the morpho-cytochemical evaluation of leukemic blasts, classified acute leukemias into myeloblastic with 7 sub types (M0-M7) and lymphoblastic with 3 subtypes (L1-L3) generally well accepted, but fails to identify prognostically relevant subgroups [5-7].

Flow cytometry has many immunophenotypic markers which in addition to detection of specific antigens also have prognostic and therapeutic implication even within single acute leukemia subtype [2].

Also Flow cytometric immunophenotyping help in deciding therapy with monoclonal antibodies directed against leukemia surface antigens including CD19, CD20, CD22, and CD52.

During the last two decades immunophenotyping has been shown to be very useful in the lineage assignment of leukemic cells [8,9]. It is used in the diagnosis, classification and prognostic evaluation of acute leukemias. Objectives of this study was to compare the morphological and cyto-chemical diagnosis of peripheral blood and bone marrow sample with flow cytometric immunophenotypic diagnosis and overall agreement between them in acute leukemia's.

Materials and Methods

This study was cross sectional prospective study taken in haematological unit during July 2016 to June 2019, at Al-Thawra Teaching Hospital Sana'a which is one of the largest specialized tertiary referral and teaching hospitals in the country.

All consecutive adult patients diagnosed with acute leukemias were analyzed before treatment. The cyto–morphological diagnosis was based on French-American-British criteria (FAB classification) [6,7] in blood and bone marrow films stained by leishmann's stain. The immunophenotyping by flow cytometry with a panel of Monoclonal Antibodies specific to acute leukemias usually used (29) as: CD3, CD5, CD7, CD10, CD13, CD 14, CD19, CD20, CD33, CD34, CD117, CD 45, CD 46, HLA-DR, Cy-MPO and TdT.

T-cell ALL: cytoplasmic (cy) CD3, CD5, CD7 For B-cell ALL: CD19, CD10 and CD 20.

Myeloid cells: CD13, CD33, CD117, CD14, CD64 and cytoplasmic myeloperoxidase (Cy-MPO).Pan leukocyte marker: CD45.

Precursor markers: CD34, TdT, HLA-DR. Any antigenic marker was considered positive if 20% or more of the blast cells reacted with a particular antibody.

We included all patients both sexes if his/her age aged.

≥ 17 years old with diagnosis of acute leukemia proved clinically, cyto-morphology and flow cytometric immunophenotyping were included in the study. Patients <17 years old, patients without complete data and who refused to participate were excluded from this study.

Procedure

Usually, the reticulocyte reference range is reported as a percentage of the red blood cells population; after the first few months of life, the normal reticulocyte percentage in children is equal to the adult's one, approximately 1.5 percent [2]. However, in patients with anemia the reticulocyte percentage must be interpreted in relation to the reduced number of red blood cells. The Absolute Reticulocyte Count (ARC), defined as the number of reticulocytes/µL, better reflects bone marrow function and effective erythropoiesis.

Statistical Analysis

All collected data were checked, edited analyzed by using computer based SPSS (Statistical Package of Social Science, version 20) software. Results were presented in the form of tables. Descriptive analysis of all relevant variables was done by using proportion, central tendency and dispersion. P value was calculated by Chi square test, P-value <0.05 was considered significant.

Results

A total of 200 acute leukemia were involved in this study. Acute leukemia was diagnosed on clinical history, morphologically, cytochemistry and flow cytometry immunophenotyping. 118 (59%) of the patients were males and 82 (41%). were females. Their mean age was (22 ± 5.56 SD years) and the younger age group (28-38 years old) was the most affected (Table 1). The ages of the patients ranged from 17 to 71 years with a mean age (22 ± 5.56 SD years). The most affected age group was 28-38 years which representing 48% of cases. While oldest group 61-71 years representing low percentage 4% of patients (Table 1).

Table 1. *Age and gender distribution of the patients with acute leukemia.*

Variable	Frequency (n)	Percent (%)	Cumulative percent
Gender			
Male	118	(59)	59
Female	82	(41)	100
Age group (years)			
17-27	24	(12)	12
28-38	96	(48)	60
39-49	50	(25)	85
50-60	22	(110)	96
61-71	8	(4)	100
Mean age ± SD	22 ± 5.56 SD		
n=200			

Ch2, 2.11 p value 0.145

Based on the morphology, of these 200 cases, 134 were classified as AML versus 126 on cytometry, while 50 were classified as ALL on morphology versus 74 in cytometry. However, sixteen cases remained unclassified by morphology (Table 2).

Table 2. *Morphological and flow cytometric immunophenotyping pattern of acute leukemia cases.*

Type of leukemia	Type of test			
	Morphological No.	(%)	Cytometric No.	(%)
Acute Myeloblastic Leukemia (AML)	134	(67)	126	(63)
Acute Lymphoblastic Leukemia (ALL)	50	(25)	74	(37)
Undifferentiated AL	16	(8)	0	(0)
Total	200	-	200	-

Immunophenotypic pattern of acute myeloblastic leukemia is shown in Table 3. Among the 126 cases of AML, highest expression rate of the markers were found in CD117, CD34, HLA-DR, CD14 and CD 45. They were positive in all patients. Followed by CD13, cy-MPO and CD33, positive in 92.06%, 85.71% and 77, 77% respectively.

Table 3. *Immunophenotypic pattern of acute myeloblastic leukemia (n=126).*

Markers	No.	%
CD13	116	92.06
CD33	98	77.78
CD117	126	100
Cy-MPO	108	85.71
CD34	126	100
HLA-DR	126	100
CD14	126	100
CD64	126	100
CD 45	126	100

Regarding ALL cases, 54 (72.97%) patients were B-ALL and 20 (27.03%) patients were T-ALL. All cases of B–ALL were positive for CD20. Flowed by CD 34 and TdT positive in 24 (32.43%) and 20 (27.03%) respectively (Table 4). All patients with T-ALL were positive for CD 19. Flowed by TdT and CD 10 positive in 28 (37.84%) and 22 (29.73%) respectively.

Table 4. *Immunophenotypic pattern of patients with acute lymphoblastic leukemia (ALL) (n=74).*

	B-ALL* 54/74 N (%)	T-ALL* 20/74 N (%)
CD34	44 (45.46)	2 (2.70)
TdT	40 (54.05)	18 (24.32)
HLA-DR	36 (48.65)	0
CD19	54 (72.97)	20 (54.05)
CD 20	54 (72.97)	0
CD10	30 (40.54)	22 (29.73)
CD 7	36 (48.65)	20 (27.03)
CD 5	32 (43.24)	12 (16.22)
CD 3	26 (35.14)	10 (13.51)

ALL*=Acute lymphoblastic leukaemia.

Out of 134 patients with AML diagnosed by Cyto-morphology, 122 patients were concordantly confirmed to have AML by flow cytometry and 12 were disconcordantly confirmed as ALL (Table 5). Out of 50 patients with ALL diagnosed by Cyto-morphology, 46 were concordantly confirmed to have ALL by flow cytometry and 4 were disconcordantly confirmed as AML. All unclassified AL confirmed to be ALL by FCA.

Table 5. *Concordance and discordance between cyto-morphology results and flow cytometry results.*

	Type of acute leukaemia			
Concordance/ discordance	**Acute Myeloblastic Leukemia (AML)**	**No. (%)**	**Acute lymphoblastic leukemia (ALL)**	**No. (%)**
Complete concordance	122	(91.04)	46	(92)
Discordance	12	(8.96)	4	(8)
Total	134	(100)	50	(100)
Partial concordance/ unclassified	-	-	16	-

Discussion

The FAB classification for acute leukemias has been the major system of classification for more than 20 years [6,7]. This system provided structured criteria for the diagnosis of a variety of morphologic and cytochemical subtypes of acute leukemias. However, studies indicate that the majority of categories in the FAB system do not delineate significant disease groups based on morphology and cytochemistry in terms of patient survival WHO in 2008 [10,11], incorporated immunophenotyping along with other parameters for best patient outcome. Thus, immunophenotyping has become an important and sensitive tool contributing with clinical, morphological, cyto (chemical and cytogenetic analyses, to diagnosis, classification, prognosis, and disease monitoring of acute leukemias [12]. This study included 200 adult patients with acute leukemia diagnosed by cyto-morphology. Immunophenoltyping was carried out by flow cytometry (FCM) from peripheral blood or bone marrow samples. The study showed that the mean age of the patients was 25 ± 5.56 SD. Which is younger than the mean age (35-47) years reported by other study [13]. This differences may be related to patients whom included in the study. We included patients with age of ≥ 17 years old. The ratio of male to female was 1.4:1 indicating a slight male predominance which is similar to some other international studies [14,15]. In this study the majority of acute leukemia cases were diagnosed by flow cytometry, was AML compared to ALL. This observation was reported before from the Middle East countries [16]. However others studies reported that ALL was predominant finding. This because our study involved only adult patients where the prevalence of AML are high among them. Subtype B (ALL in our study population found to be more frequent than subtype T-ALL (72.97% versus 27.03%). This result coincide with studies done before, where sub type of B (ALL was predominant finding. Expression rate of CD117 in our study was found in all patients with AML similarly, the CD13 was the second frequent recognized marker found in (92.06%) of the patients. Nearly similar result was reported from other studies [17].

These markers are specifically useful for the identification of morphologically undifferentiated AML cases. Avery crucial marker for diagnosis of myeloid leukemia is Cytoplasmic Myeloperoxidase (cy (MPO), which was detected in 85.71% of the patients, higher than a study done in 2000 that showed positivity in 73% (19). This may be due to small sample size in this study. Hematopoietic progenitor cell markers CD34 and HLA (DR in acute myeloid leukaemia were expressed in our cases in all of AML which was corresponded to other study [18]. Expression of CD20 in this study found in all cases of B (ALL which is inconsistent with study in Egypt where CD19 reactivity found in all B-ALL [16]. Variation of CD34 between AML and ALL was detected, it was found in all patients in the former and it was only identified in 40% of our patients presented with ALL. CD34 is normally expressed in immature haemopoitic cells or blasts so it is an excellent marker for monitoring blast population. However, variable expression rate of CD34 was found in different studies. In this study Expression of TdT in T (ALL cases was lower than studies done in other region. However, Lahjouji et al in Morocco reported that the expression of TdT in adult T (ALL was found to be 50%, which was similar to the current study. This discrepancy may be due to variable maturity of T (lymphoblast in T-ALL, because the expression of TdT decrease by maturation. Or it may be related to variation in sample size of the study giving a false impression [17-25].

Complete lineage agreement between cyto(morphology and flow cytometry were 91.04% for AML and 92% for ALL. The overall concordance between morphology and flow cytometric findings in acute leukemias was 168 (84%). Discordant results were found in 16(8%) cases where flow cytometry was essential for lineage assignment. Partial concordance or unclassified was considered when blast type could not be confirmed morphologically and was diagnosed as ALL by flow cytometry found in 16 (8%). Similar results were reported by other studied [25-29].

Conclusion

Flowcytometric immunophenotyping could precisely delineate different forms of Acute Leukemia and is especially important for confirming cyto (morphologically undetermined acute leukemia. AML was the predominant form of leukemia in adults aged more than 17 years. CD13 and CD 117 were the mostly positive marker for AML. While for B (ALL, CD20, CD34 and TdT, were positive and for T (ALL CD19, TdT and CD10 were positive).

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Ethical Consideration

Permission and approval of hospital committee was obtained and consent was taken from each participant. All data were dealt with confidentiality.

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***Correspondence to:**

Ahmed Kiad Salem
Department of internal medicine
Sana'a University
Sana'a
Yemen
E-mail: ahmidkaid@yahoo.com
Tel: 00967733760019/00967772535053