Flow Cytometric Analysis of Acute Leukemia Cases in Aseer Area of the Kingdom of Saudi Arabia

Ghada Rayyan[1], MSc., Mohammad Shubair[2], PhD., Mubarak Asiri[3], MBBS, MD FRCPA.

Abstract: Objective: To determine and to estimate the occurrence of acute leukemia's in Aseer Area, Saudi Arabia And to identify the common immunophenotypes of acute leukemia using flow cytometric analysis.

Methods: Thirty five patients were included in this descriptive study which was conducted at the Armed Forces Hospital in collaboration with Aseer central Hospital during the period from January 2009 to January 2010, peripheral blood and bone marrow samples were collected from those patients suspected with acute leukemia. Laboratory investigations comprised blood and bone marrow films, complete blood count as well as flow cytometric analysis. Data were analyzed using the SPSS 15 statistical package.

Results: Thirty five cases were diagnosed as acute Leukemia ones and were classified as Acute Myelogenous Leukemia (AML), n=13(37.1%) and Acute Lymphoblastic Leukemia (ALL), n=22 (62.9%). Their diagnosis was determined according to the standard French- American- British (FAB) criteria in addition to immunophenotyping. Leukemia was diagnosed among adults in 68.6% whereas among children with age less than or equal to 12 years in 31.4%. The patient''s age ranged from one year to 74 years with a mean of 26.5 years. Twenty one(60%) were males and 14 (40%) were females with a male:female ratio of 1.5:1. For ALL the most common immunophenotype was Pre-B ALL (63.6%), followed by Pre-T ALL (31.8%), and B-ALL (4.5%). For AML it was found that M1/M2 (38.5%), M4/M5 (30.8%), M3 (23.0%), and M0 (7.7%) were detected.

Conclusion: Immunophenotypic studies are essential to distinguish acute lymphoblastic leukemia (ALL) from minimally differentiated acute myeloid leukemia (AMLM0) and to classify ALL into immunologic subtypes. Supporting conventional methods(CBC, blood and bone marrow films) with Flow cytometry proved to be important for final diagnosis as flow cytometry was found to be especially useful in the correct identification of AML M0 and differentiation of acute promyelocytic leukemia (APL) from AML M1/M2. It is recommended to conduct more research to determine genotypes and phenotypes of different leukemia markers.

Keywords: Acute Leukemia, Immunophenotyping, Flow cytometry. Saudi Arabia.

1- Senior technologist, The Islamic University, Gaza, Palestine.
2- Professor of Immunology(corresponding author), The Islamic University, Gaza, Palestine. E. Mail: mohshubair@gmail.com
3- Consultant hematologist and medical director of Armed Forces Hospital, Southern region, Saudi Arabia

الكشف عن حالات سرطان الدم الحاد بواسطة تقنية التدفق الخلوي
في منطقة عسير بالمملكة العربية السعودية

ملخص: **الهدف**: معرفة مدى انتشار مرض سرطان الدم الحاد في منطقة عسير بالمملكة العربية السعودية وللتعرف على المكونات المناعية للخلايا السرطانية باستخدام تقنية التدفق الخلوي.

الطريقة:

تعتبر هذه الدراسة دراسة وصفيه وقد أجريت في مستشفيات القوات المسلحة بمنطقة عسير بالتعاون مع مستشفى عسير المركزي خلال الفترة من يناير 2009 إلى يناير 2010 وقد تم الحصول على عينات الدم ونخاع العظام من المرضى المصابين. وقد شملت التحاليل ألمخبريه عد الدم الكامل، أفلام للدم ونخاع العظام بالإضافة إلى استخدام تقنيه التدفق الخلوي. وتم تحليل النتائج إحصائيا باستخدام برنامج SPSS 15.

النتائج:

تم تشخيص 35 حاله من المصابين بسرطان الدم الحاد وكان من بين هذا العدد 13 حاله مصابه باللوكيميا النخاعية الحادة (AML) بنسبة 37,1% كما كانت 22 حاله مصابه باللوكيميا الليمفاويه الحادة (ALL) وبنسبة 62,9%. وقد تم تأكيد التشخيص اعتمادا على المعيار الفرنسي الأمريكي البريطاني لتقسيم مرض سرطان الدم الحاد بالاضافة إلى الكشف عن المكونات المناعية لخلايا الدم المصابة. وقد كانت معظم الحالات عند البالغين بنسبة 68,6% بينما كانت النسبة عند الأطفال 31,4%. وقد تراوحت أعمار المرضى من سنه واحده إلى 74 سنه بمتوسط 26,5 سنه. وكانت نسبة الذكور 60% والإناث 40%. وقد كان المكون المناعي الشائع عند المرضى المصابين باللوكيميا الليمفاويه الحادة هو Pre-B ثم يليه Pre-T ثم B-mature وكانت المكونات المناعية الأكثر شيوعا عند المرضى المصابين باللوكيميا النخاعية الحادة هي M1-M2 ثم M4-M5 ويليها M3 ثم M0.

الاستنتاج:

أظهرت هذه الدراسة أن تعزيز الطرق التقليدية (عد الدم الكامل، أفلام الدم ونخاع العظام) بالكشف عن المكونات المناعية للخلايا السرطانية بواسطة تقنية التدفق الخلوي له أهميه كبيرة في تشخيص الأنواع المختلفة من سرطان الدم الحاد مثل M0 وكذلك M3 وتمييزها عن باقي الأنواع الأخرى من اللوكيميا الليمفاوية الحادة ويوصي الباحثون بإجراء المزيد من الأبحاث لتحديد النمط الجيني والمظهري لخلايا الدم المستهدفة مما سيساعد في تحديد نظام العلاج المناسب.

Introduction

Acute and chronic hematological malignancies, or "blood cancers" (leukemia's, lymphomas, and multiple myeloma), form a distinct subset of cancers that originate in the bone marrow or in the lymph nodes[1]. Leukemia results from the proliferation of a clone of abnormal hematopoietic cells with impaired differentiation, regulation, and

Flow Cytometric Analysis of Acute Leukemia Cases

programmed apoptosis(2). Leukemia's account for approximately 8% of all human cancers, and approximately half of these cases are classified as acute leukemia. Up to 90% of acute leukemia's are in form of lymphoblastic leukemia, ALL which is characterized by the abnormal growth of lymphocyte precursors, the lymphoblasts. Acute myelogenous leukemia (AML) (also known as acute nonlymphoblastic leukemia, or ANLL) causes the rapid proliferation of megakaryocytes, monocytes, granulocytes, and RBCs(3). The acute leukemias are characterized by aberrant differentiation and proliferation of malignantly transformed hematopoietic stem cells. These cells accumulate within the bone marrow and lead to suppression of the growth and differentiation of normal blood cells(2). Symptoms result from varying degrees of anemia, neutropenia, and thrombocytopenia or from infiltration into tissues. Although virtually any organ system may become involved once leukemia cells enter the peripheral blood, the lymph nodes, liver, spleen, central nervous system (CNS), and skin are the most common sites detected clinically(4). Acute leukemia is a rare form of cancer that affects both children and adults (5) with an incidence rate of 4/100,000 per year in the developed countries(6).

The diagnosis of acute leukemia entails a stepwise approach. First in sequence and importance is the distinction of acute leukemia from other neoplastic diseases and reactive disorders. Second is differentiating AML and ALL and, third, facet is the classification of AML and ALL into categories that define treatment and prognostic groups(7). Immunophenotyping is a widely used method to diagnose and classify acute leukemias, thereby complementing morphology and cytochemistry. A variety of clinical and biological parameters, including immunophenotype, have been examined for potential value in predicting treatment response and survival(8). Flow cytometric techniques are widely used in clinical hematology(9). Characterization of leukemia's by immunotyping is particularly helpful when the morphology is difficult to interpret.The major advantage of using immune markers by flow cytometry is the identification of particular leukemia subtype, not recognized by morphologic criteria, which may have prognostic significance. Immunotyping of peripheral blood (PB) and bone marrow (BM) in leukemia's does not only determine the decision making for a specific therapeutic regimen, but also is a practical prognostic indicator(9). The diagnosis and management of AL largely depends on the detection, identification and characterization of leukemic cells. Traditionally, applications of flow cytometry in leukemia diagnosis have relied on the assignment of the blast cell lineage. The identification of a T, B or myeloid origin of leukemic cells is currently straightforward in

most occasions. However, it has become evident that each AL subgroup has heterogeneous biologic characteristics, of which many are associated with a different response to therapy(10). A number of observations have emerged showing strong association between specific immunophenotypes and genetic recurrent abnormalities underlying the malignant transformation, with prognostic value(11).   Although many acute leukemia's can be correctly identified morphologically with or without enzyme cytochemical analysis, immunophenotyping remains indispensable for proper identification of myeloid lineage in minimally differentiated acute myeloid leukemia (AML M0) and determination of B-cell or T-cell lineage in acute lymphoblastic leukemia (ALL). Also, expression patterns of CD34 and HLA-DR help in distinguishing acute promyelocytic leukemia (APL/AML M3) from AML M1/M2(12).

Material and Methods.

Thirty five patients referred to the Forces Hospital and Aseer Central Hospital, KSA, were subjected to laboratory investigations within the period between January 2009 and January 2010. The patients were suspectedly having acute leukemia. Peripheral blood and bone marrow samples were collected from those patients. Hematological diagnosis was based on morphology and immunophenotyping.

Bone Marrow Samples: Samples of bone marrow were obtained from the sternum, iliac crest or anterior or posterior iliac spines. The specimens were collected by physicians and delivered into Na-Heparin tube or EDTA tube..

Sample preparation:

Peripheral blood samples

- Patients WBC count was adjusted at no more than 10x 10^3/ml. Any specimen which was having more than this count was diluted until reaching the proper count. The specimen was rejected if it contained any clot. A blood film was prepared for microscopic examination.

Bone marrow samples

- A bone marrow film was prepared for microscopic examination. The specimen was checked for clot presence, if any was present, it was disintegrated with wooden sticks. The specimen was washed before processing to remove any fat or proteins(13). Washing was performed by adding about 3-4 ml of 2% PBS to 1-1.5 ml of specimen in test tube. Then the tube was centrifuged for 5 minutes at 3500 rpm, the supernatant was aspirated and the pellet was resuspended with adequate volume of 2%PBS and washing was repeated. After washing, cell count was performed on CBC analyzer.

Flow Cytometric Analysis of Acute Leukemia Cases

Lysing and Staining.
 The appropriate volume of fluorochrome-conjugated monoclonal antibody was added to 100µL of whole blood in a 12 x 75-mm tube, 20µl of fluoresceineisothiocyanate(FITC), 20µl of Phycoerythrin(PE), 5µl of Allophycocyanin(APC), and 5µl of Peridinin-chlorophyll protein complex(perCP). The mixture was Vortexed gently and incubated 15 to 30 minutes in the dark at room temperature (20° to 25^oC). A volume of 2 ml of 1 X FACS lysing solution was added. Then it was vortexed gently and incubated for 10 minutes in the dark at room temperature and Centrifuged at 500 x g for 5 minutes. The supernatant was removed.
  A volume of 2 to 3 mL of wash buffer was added and centrifuged at 500 x g for 5 minutes. The supernatant was removed. A volume of 0.5 mL of 1% paraformaldehyde solution was added and mixed thoroughly, and then it was stored at 2° to 8° C until analyzed.
Analysis on a FACS brand flow cytometer. Samples were mixed thoroughly before acquisition.

Flow Cytometry:
The (Fluorescence Activated Cell Sorter is a trademark of Becton, Dickinson and Company, CA, USA) BD FACS Calibur ^{TM} system is a four-color, dual-laser, bench-top system available today that is capable of both cell analysis and sorting. It is designed specifically to support a wide range of applications, the BD FACSCalibur system is a fully integrated multiparameter system.
Lasers: Air-cooled argon-ion laser, 15 mW 488 nm. Red diode laser: nominally 635 nm. Emission Wavelengths: FLC1: 530/30 (Laser 1), FLC2: 585/42 (Laser 1), FLC3: 670 LP (Laser 1), FLC4: 661/16 (Laser 2).
Sample Flow Rates: Three selectable flow rates of 60 µL/min, 35 µL/min, and 12 µL/min. Pressure difference between sheath and sample was regulated and monitored; particle velocity in flow cell was approximately 6 meters/sec. The concentration for single-cell suspensions was $10^5$to 2 x 10^7particles/mL. The range of particle sizes that can be used on this machine was 0.5µm to 100µm. It is important to ensure that there were no oversized particles (>100µm) in samples. Cell preparations with oversize were pre-filtered with a 100µm screen if necessary.
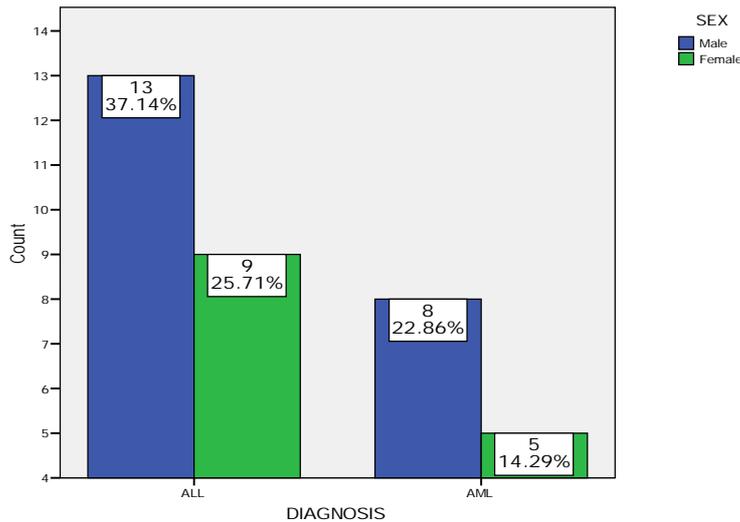
Acute Leukemia Panel:
CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11b, , CD11c, CD13, CD14, CD15, CD16+56, CD19, CD20, CD22, CD33, CD34, CD41, CD45, CD56, CD57, CD58, CD71, CD117, Kappa, Lambda, HLA-DR, Cy-CD3, Cy-CD79a, Cy-CD22, MPO and TdT.

Statistical analysis

Data were analyzed qualitatively and quantitatively using the Statistical package for social sciences (SPSS 15). Frequency and descriptive analysis was used in all statistical process. *Pearson/Spearman* correlation coefficient and *Mann-Whitney test* were used to determine any significant relationship between two variables. The Chi-square test was also used to determine the relationship between two categorical data. A *p* value of <0.05 was accepted to indicate statistical significance.

Results.

Out of 35 acute leukemia cases, 13 (37.1%) and 22 (62.9%) were diagnosed as AML and ALL, respectively. Thirteen (37.1%) and 9 (25.71%) of those having ALL while 8(22.8%) and 5(14.3%) of those having AML were males and females respectively. No significant (P =0.866) correlation between sex and diagnostic findings could be concluded based on Chi-square test(**Figure 1**).


Figure 1- Distribution of the study sample according to sex and diagnosis of acute leukemia

Most of cases were adults(68.6%). Children less than or equal to 12 years represented 31.4%. The age of patients ranged from one year to 74 years with a mean age of 26.5 years. There were 21 (60%) males and 14 (40%) females. The male to female ratio was 1.5:1. Age distribution is shown in

Flow Cytometric Analysis of Acute Leukemia Cases

Figure 2.
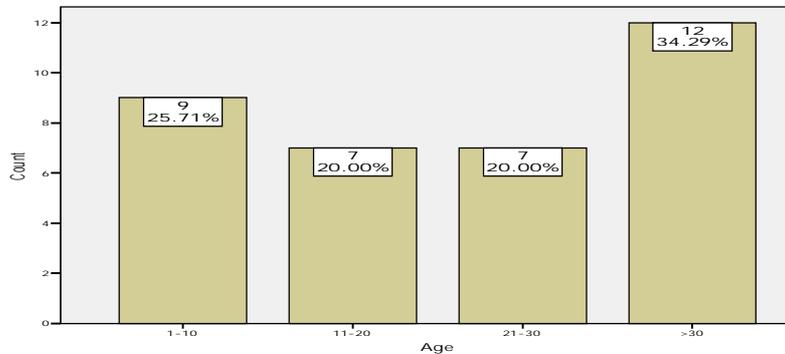
Figure 2- Distribution of age in acute leukemia.

The percentages of ALL for pediatrics and adults were 28.57% and 34.29%, respectively while that of AML for pediatrics and adults were 2.86% and 34.29%, respectively. The results obtained by the Chi-square test indicate a significant (P =0.020) correlation between age and defined diagnosis. The age group categorization of acute leukemia patients are given in **Figure 3**.
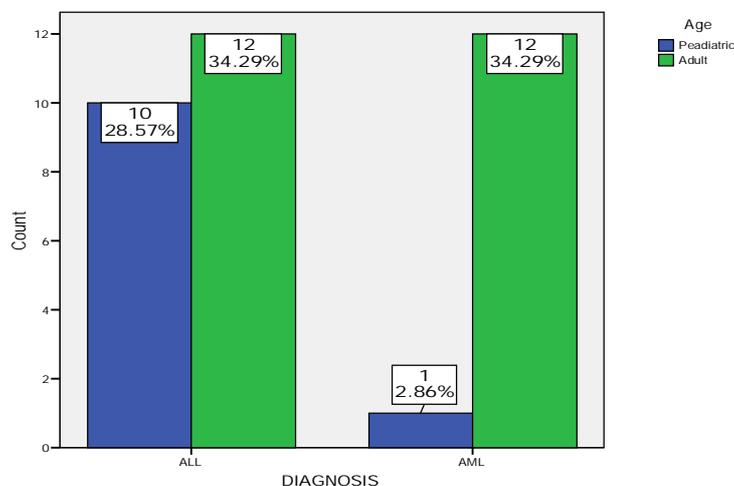
Figure 3- Group categorization of acute leukemia patients.
The studied markers were myeloid and monocytic markers (*i.e.,* CD33, CD13, CD14, CD45), lymphoid markers (*i.e.,* CD2, CD3, CD5, CD7, CD19, CD20, CD22), independent markers (*i.e.,* CD10, CD34, HLA-DR) and cytoplasmic enzyme TdT, and MPO. The most common lymphoid

markers in patients with T- cell ALL were CD2 (57.1%), CD3 (100%), CD5 (85.7%), CD7 (85.7%), TdT (71.4%) and in those with B-cell ALL were CD10 (100%), CD19 (100%), CD22 (86.67%), CD79a (100%) and HLA-DR (93.3%). The most common ALL subgroups that express myeloid markers were CD13 (9.09%) and CD33 (4.54%). Among different markers, the most positive markers in myeloid lineage antigens were CD13 (100%), CD33 (92.3%), and MPO (66.7%) and the hematopoietic progenitor cell markers HLA-DR (69.2%), CD117 (75.0%), CD34 (76.9%), and CD45 (100%). There were aberrant lymphoid antigen expressions in acute myeloid leukemia. CD7, a stem cell marker which was positive in 7.7% of patients in M1/ M2. We detected T-cell markers CD2 in 7.7% of patients in M4/ M5. The subclassification of acute lymphoblastic leukemia and acute myelogenous leukemia are shown in **Table 1**and **Table 2**.

Table 1- Immunophenotyping classification of ALL..

Immunophenotyping	Cases	Percent %
ALL	22	62.9%
Pre-B ALL	14	63.6%
Pre- T ALL	7	31.8%
B-ALL	1	4.5%
Null (early pre-B) ALL	0	0.0

Table 2- Immunophenotyping classification of AML.

Immunophenotyping	Cases	Percent %
M0	1	7.7%
M1- M2	5	38.5%
M3	3	23.0%
M4 - M5	4	30.8%

The CBC analysis showed a wide range of variations ranging from subnormal to normal in Hemoglobin concentration and Platelets.. The Leukocyte count also varied from leucopenia to hyper leukocytosis. **Table 3** shows the normality test for each variable of ALL and AML.

Flow Cytometric Analysis of Acute Leukemia Cases

Table 3- The normality test of CBC according to Shapiro-Wilk Test of Normality

Variable	Diagnosis	
	ALL	AML
WBC	0.074	0.687
Hemoglobin	0.110	0.155
Platelet	0.102	0.002*
Neutrophil %	0.385	0.605
Lymphocyte%	0.008*	0.041*
Monocyte %	0.043*	0.001*
Eosinophil %	0.000*	0.003*
Basophile %	0.000*	0.000*
Blast%	0.002*	0.147

* The distribution is not normally distributed.

Descriptive statistics for the variables of acute lymphoblastic leukemia. The WBC ranged from (0.80 - 112.00x10^9/L) with median of 7.44x10^9/L. The mean values and range for hemoglobin was 8.06gm/l (4.80 - 9.96gm/l), and the platelet count range was from (11- 477x10^9/L) with median of 32.10x10^9/L.

Descriptive statistics for the variables of acute myelogenous leukemia. The WBC ranged from (1.21 - 104.00x10^9/L) with a median of 37.20x10^9/L. The median and range for hemoglobin was 9.0gm/l (4.60 – 17.40gm/l), and the platelet count range was from (9 - 279x10^9/L) with a median of 24.20x10^9/L.

Discussion:

Immunophenotyping allows for rapid and reproducible diagnosis in the majority of cases of acute leukemia[14]. It is of particular importance in recognizing the major immunologic subclasses of acute lymphoblastic leukemia (ALL), and in identifying subtypes of acute myeloblastic leukemia (AML) which cannot be differentiated by morphology and cytochemistry alone, such as FAB M0 or M7 **[15].** Flow cytometric analysis of acute leukemia is interpretive, combining the patterns and intensity of antigen expression to reach a definitive diagnosis **[14].**

Male to female ratio in present study is 1.5:1 which is in concordance with that reported in national and international studies[16]. However this is similar to mean age reported in these studies from Riyadh, Saudi Arabia and Pakistan[17,18,19]. The differences in mean age between those recorded in the present study and in western studies may be due to the exposure of different environmental factors and occupational hazards. These may

include pesticides, food preservatives, exposure to gasoline,diesel, motor exhausts, hair dyes, medications as well as genetic abnormalities.

There was a predominance of males among both children and adults. This difference between male and female ratio may be attributed to the fact that males are more exposed to many factors such as industrial, agricultural, petrol stations, which are benzene emitting sources.

There are other results which are similar to our results e.g. In Karachi, and in Northwestern Iran(20), However, in these results, In AML the age of patients ranged from three years to 74 years with a mean age of 39.5 years. The male to female ratio was 1.6:1. But in ALL The age of patients ranged from one year to 62 years with a median age of 18.1 years, 59.1% were males and 40.9% were females. The male to female ratio was 1.4:1. The similarity of our results to others might be due to the use of similar techniques and the patients were exposed to the same environmental conditions.

According to the results of immune markers, it was found that 31.8% suffering T ALL and68.2% suffering B ALL. In T ALL the ratio of pediatric to adults was 3:4 and in B ALL the ratio was 1:2 respectively. The sex distribution was 22.73% for males and 9.09% for females in T ALL, and 36.36% and 31.82% in B ALL respectively.

Acute lymphoblastic leukemia is a major subtype of leukemia in children, where as AML is as common in adults **(21).**

It is well known that the immunity of pediatrics is weak which may the cause of higher prevalence of ALL among them.

T-cell ALL represents approximately 15% to 20% of all cases of ALL in Western countries. Only a small subset of these tumors occur in adults, and this disease is less common with increasing patient age, being truly rare in patients exceeding 60 years of age. Owing to its relative rarity, T-ALL arising in adults, particularly in elderly people, has not been studied extensively. While older age has been associated with worse prognosis in ALL, most of these studies have focused predominantly on precursor B-cell ALL, which is far more common than T-ALL in the elderly. Furthermore, although it is well documented that precursor B-cell ALL occurring in elderly people (ie, older than 60 years) has a poor prognosis, possibly related to a relatively high prevalence of the Philadelphia chromosome **(22)**.

Immunophenotyping of acute leukemia **in Northern India** plays an important role in management and understanding of acute leukemia(23). In the West, the predominant immunophenotype observed in ALL in children is c-ALL, accounting for 60-80% of cases whereas T-ALL comprises only

Flow Cytometric Analysis of Acute Leukemia Cases

11-20%. In contrast, earlier studies from other countries like Japan, and South Africa have shown higher incidence of T-ALL.

Previous studies from India have reported a lower incidence of c-ALL and a higher proportion of T-ALL. This high frequency of T-ALL has been attributed to the poor socio-economic status, poor environmental conditions and low levels of health awareness.The leukemia's were classified into B-lineage ALL if the expression of the CD marker was seen in >20% of total cells for CD10, CD19, HLADR, and T-lineage ALL if CD marker was positive (seen in >20% of total cells) for CD2, CD3, CD5, CD7, CD4, CD8 **(23).**

The incidence of AML increases with age and was more frequent above 60 years. This was probably due to increasing environmental exposure to carcinogens and weakened immunity.

In Piauí of Brazil. AML occurred predominantly in adults (77%), with a median age of 34 years, similar to that found in the southeast of Brazil but lower than the median age in the United States and Europe ,52 years(24).

FAB distribution was similar in children and adults and FAB-M2 was the most common type, as also found in Japan. The high frequency of FAB-M3 described in most Brazilian studies and for Hispanics in the United States was not observed. ALL presented the characteristic peak of incidence between 2-8 years. Most of the cases were CD10+ pre-B ALL. In 25%, abnormal expression of myeloid antigens was observed. Only 10% of the patients were older than 30 years. Overall survival was better for children. Due to the effectiveness of therapy and remission of immunity **(24).**

M2 was more common in Japan than in Australia, whereas M4 occurred more frequently in Australia than in Japan. Immunophenotypically, Japanese M4/M5 more frequently displayed CD13 and CD14 than Australian, whereas the stem cell markers, CD34 and HLA-DR were observed at a relatively higher rate in Australian M3 than in Japanese M3. The B cell antigen, CD19 was more frequently seen in Japanese M2 than in Australian M2, but found more often in Australian M5 than in Japanese M5. In both populations, a close relationship was observed between the expression of CD19 and t(8;21). These findings suggest different biological characteristics of AML between the two populations, the main differences being generated by a higher frequency of t(8;21) chromosomal abnormality in Japanese AML **(25).**

These findings emphasize the fact that each population has its own characteristics which are based on hereditary and environmental factors.

In Tainan, Taiwan. Acute Myelomonocytic Leukemia diagnosed with flow cytometric immunophenotyping, the blasts expressed CD13, CD33, CD117,

myeloperoxidase and CD34 **(26).** In fact this result is in agreement with our result. Both populations may have been exposed to the same mutagens.

The FAB distribution of AML has been extensively studied in the past decades at national and international levels. Most published data indicate the predominance of M2 as a most common subtype. Occurrence of this subtype is also common after primary malignancy**(16).** However,Nakase et, al **(25)** showed AML-M4 as common subtype in Australian population compared to Japanese, where AML-M2 is common. Present studies also confirm M1-M2 as the most common type followed by M4-M5.

Many of the differences in AML subtypes may be due to the subjectivity of morphologic diagnosis together with variable nature of acute myeloid leukemia subtypes, with no real demarcation. Some genetic factors may be responsible for particular FAB subtypes of AML in our population. Secondly most studies at national level have small number of patients and probably with underutilization of cytochemical stains. The other reason for this discrepancy may be patients of different ethnic group and or geographical variation**(16).**

Flow cytometry thus had a role to play in ALL patients to confirm a definite and a probable diagnosis, to define therapeutically and prognostically groups such as B and T lineage ALL and to distinguish AML – M0 from ALL.

Flow cytometric method helps in diagnosing AML cases as well, but is a less essential mode of investigation in this group purely from the perspective of the therapy regime. But its role in defining different subgroups in AML is its major use. While morpho-cytochemistry provides a first-line investigation of great therapeutic value, and more so in AML, it needs to be supplemented by flow cytometry, particularly in ALL **(27).**

Flow cytometric (FC) immunophenotyping is considered a powerful ancillary tool for diagnosing APL. Leukemic promyelocytes characteristically express mature myeloid markers, including CD13 and CD33, and are negative for CD34 and HLA-DR. However, this immunophenotype is not specific for APL. Furthermore, the typical immunophenotype commonly is associated with M3, whereas M3v is more heterogeneous, with variable expression of CD2, CD34, CD56, HLA-DR, and terminal deoxynucleotidyl transferase **(28).** From our results there was one case APL which had CD79a expressed in AML.

Acknowledgment

Flow Cytometric Analysis of Acute Leukemia Cases

References:

1. -**Mucke H., (2005) -** Hematological Cancer Therapeutics: Pipelines and Competition. C H A, P.1-88, available at: www.advancesreports.com
2. -**Greenberg M., Glick M., and Ship J., (2008)** - Burket's Oral Medicine. 11th Ed. Chapter 16, BC Decker Inc, Available at: http://online.statref.com
3. -**Sommers M., Johnson S., and Beery T., (2007)** - Disese and Disorders: A Nursing Therapeutics Manual. 3rd Ed, Chapter. Leukemia, Acute, F. A. Davis Com, available at: http://online.statref.com
4. -**Devine S., and Larson R., (1994) -** Acute Leukemia in Adults: Recent Developments in Diagnosis and Treatment. C A- J Clin, Vol.44, No.6, P. 326 - 352.
5. -**Rauscher G., Sandler D., Poole C., Pankow J., Mitchell B., Bloomfield C., *et al*., (2002)** - Family History of Cancer and Incidence of Acute Leukemia in Adults. Am J Epidemiol, Vol.156, No.6, P. 517–526.
6. -**Steffen C., Auclerc M., Auvrignon A., Baruchel A., Kebaili K., Lambilliotte A., *et al*.,(2004)** - Acute Childhood Leukemia and Environmental Exposure to Potential Sources of Benzene and other Hydrocarbons; A Case-Control Study. Occup Environ Med, Vol.61, P.773–778.
7. -**McKenna R., (2000)** - Multifaceted Approach to the Diagnosis and Classification of Acute Leukemia's. Am Asso for Clin Chem, Vol .46, P.1252-1259.
8. -**Slobinas A., and Matuzevièienë R., (2005) -** The immunophenotype of adults with acute myeloid leukemia: proposal of prognostic value. Acta Medica Lituanica, Vol. 12, No. 3, P. 54–59.
9. -**Rezaei A., Adib M., Mokarian F., Tebianian M., and Nassiri R., (2003)** - Leukemia Markers Expression of Peripheral Blood vs. Bone Marrow Blasts using Flow cytometry. Med Sci Monit, Vol.9, No. 8, P.359-362.
10. -**Basso G., Lanza F., Orfao A., Bene M., Borowitz M., Campana D., *et al*., (2002)** - Flow Cytometryc Immunophenotyping of Acute Lymphoblastic Leukemia is the Time Ready for Consensus Guidelines?. J Biol Regul Homeost Agents, Vol.16, P. 257-258.
11. -**hianese R., Brando B., and Gratama J., (2002)** - Diagnostic and Prognostic Value of Flow Cytometric Immunophenotyping in Malignant

Hematological Diseases. J Biol Regul Homeost Agents, Vol.16, P. 259-269.

12. -Kaleem Z., Crawford E., Pathan M., Jasper L., Covinsky M., Johnson L., *et al*., (2003) - Flow Cytometric Analysis of Acute Leukemia's. Arch Pathol Lab Med, Vol. 127, No. 1, P. 42–48.

13. -Bain B. (2001)- Bone marrow aspiration, J Clin Pathol, Vol. 54, P.657-663.

14. -Jennings C., and Foon K., (1997)- Recent Advances in Flow Cytometry: Application to the Diagnosis of Hematologic Malignancy. Blood, Vol. 90, No.8, P. 2863-2892.

15. -Xu X., Wang, J., Lu S., Chen L., Yang J., Zhang W., et al., (2009)- Clinical and biological characteristics of adult biphenotypic acute leukemia in comparison with that of acute myeloid leukemia and acute lymphoblastic leukemia: a case series of a Chinese population. Haematologica, Vol. 94, No. 7, P. 919-927.

16. -Harani MS et, al(2005)- Frequency of fab subtypes in acute myeloid leukemia patients at Aga Khan university hospital Karachi. J Ayub Med coll abbottabad ,Vol. 17, No.1, P.26-29.

17. -Khalil S., Jackson J., Pyle R., and Robichaud M.,(1995)- Immunophenotyping of acute leukemia at king FiasaL Specialist Hospital and Research Center. Ann. Saudi Med, Vol. 15, No. 2, p. 137-139.

18. -Khan A., Hussein N., Al sagh A., Malatani T., and sheikh A., (1991)- Pattern of cancer at Aseer Central Hospital, Abha, Saudi Arabia. Ann Saudi Med, Vol. 11, No. 3, P. 285-288.

19. -Khawaja MR., Allana SS., Akbarali AN., Adil SN., Khurshid M., and Perves S., (2005)- Flow Cytometric and Demographic Analysis of T Cell Acute lymphoblastic Leukemia in Pakistani population. J Ayub Med Coll Abbottabad, Vol. 17, No. 4.

20. -Keramani I., (2002)- Immunophenotyping of Acute Leukemia in North western Iran. IJMS, Vol 27, NO. 3, P. 136-138.

21 -Horibe K., Tsukimoto l., and R Ohno R.,(2001)Clinicopathologic characteristics of leukemia in Japanese children and young adults. Leukemia, Vol. 15, No. 8, P. 1256-1261.

22.- Onciu M., Lai R., Vega F., Ramos C., and Medeiros L., (2002)- Precursor T-Cell Acute Lymphoblastic Leukemia in Adults: Age-Related Immunophenotypic, Cytogenetic, and Molecular Subsets. Am J Clin Pathol, Vol. 117, P. 252-258.

23. - Sazawal S., M Bhargava M., Arya LS, Khattar A., and Saxena R,, (2008)- Acute Lymphoblastic Leukemia in Children: Changing

Flow Cytometric Analysis of Acute Leukemia Cases

Immunophenotypic Pattern in Northern India. Indian J Med & Paed Oncol, Vol. 29, No.2, P. 49-50.

24. -Rego M., Pinheiro G., Metze K and Metze l., (2003)- Acute Leukemias in Piaul: Comparison with features observed in other regions of Brazil. Braz J Med Biol Res, Vol. 36, No.3, P. 331-337.

25.-Nakase K., Bradstock K., Sartor M., Gottlieb D., Byth K., Kita K., et al,. (2000)- Geographic heterogeneity of cellular characteristics of acute myeloid leukemia: a comparative study of Australian and Japanese adult cases. Leukemia, Vol. 14, No.1, P. 163-168.

26- Chang S., Hsieh Y., Lee L., Tzeng C., and Chuang S., (2005)-Acute Myelomonocytic Leukemia with Abnormal Eosinophils: A Case Report with Multi-Modality Diagnostic Work-up. Chang Gung Med J, Vol.29, P. 532-537.

27.-Sengar M., Rai A., Saxena A,. Singh A., Raina V. Seth T., el al., (2008)- Acute leukemia: Diagnosis improved by flow cytometry in addition to morphology. Asia-Pacific J Clin Oncol, Vol.5, No.1, P. 55-65.

28.-Lin P., Hao S., Medeiros L., Estey E., Pierce S., Wang X., et al., (2004)- Expression of CD2 in Acute Promyelocytic Leukemia Correlates With Short Form of PML-RARa Transcripts and Poorer Prognosis. Am J Clin Pathol, Vol. 121 P. 402-407