Acute Myeloid Leukemia

Dr Shruti Chauhan

Dr Sachin Sharma

Dr Mayank Chauhan

Acute Myeloid Leukemia by Dr Shruti Chauhan, Dr Sachin Sharma and Dr Mayank Chauhan

Published by Pink Petals Publication Pvt Ltd.
Naya Moradabad, B-87-Sector-6, Moradabad (244001), India.

Price: 500 | $ 20
First Edition - 2024

ISBN: 978-93-94156-02-9
ISBN 93-94156-02-X

Cover image: https://www.istockphoto.com/

This book is available as a free download from https://pinkpetalspublications.com

Printer: Reprographic Services, New Delhi, India.

Preface

The book is a comprehensive overview of the topic Acute Myeloid Leukemia, the most common frequent leukemia in adults, and it's diagnosis through ancillary techniques that is immunophenotyping but at the same time, emphasising on morphology, clinical features and cyto chemistry. Detailed opinion is provided on diagnostic workup of the disease. This book would be an aid to both undergraduate and postgraduate students to have an insight into the topic. The book is written by an expert with wide teaching and clinical experience.

ACKNOWLEDGEMENT

It is sacred duty on my part to pay my debt of gratitude, from the depth of my heart, to The Almighty God, for the grace and kindness that he bestowed upon me to bring out this manuscript.

No words can express the depth of my sense of gratitude and regards to my esteemed teacher and Guide Prof. Uma Shankar Singh, Department of Pathology, King George's Medical University, Lucknow. It is only his blessings and masterly guidance from time to time which helped me to complete this work.His deep sense of understanding, incomparable power of observation and unprejudiced healthy criticism helped me understand the work in greater depth & clarity. I also thank him for permitting me to undertake this study and conduct the work successfully.

I express my sincere and deep sense of appreciation to Prof. Ashutosh Kumar,Head, Department of Pathology King George's Medical University, Lucknow, I also express my unfathomable sense of appreciation to him for extending the permission to utilize the departmental lab facilities which has helped in shaping this study in its current form.

I also wish to express my regards and gratitude to my esteemed teacher Prof. M.M.Goel, Department of Pathology, King George's Medical University, Lucknow for her inspiration, constant encouragement and invaluable advices throughout this study.

I convey my sincere thanks to my esteemed teacher Prof. A K Tripathi, Department of Hematology, King George's Medical University, Lucknow, for his understanding, inspiration and support throughout this study.

I convey my sincere thanks to my esteemed teacher Prof. Archana Kumar, Department of Pediatrics, King George's Medical University, Lucknow, for her motivation, encouragement and invaluable advices throughout this study.

I am greatly obliged & thankful to my esteemed teacher, Dr Rashmi Kushwaha, Department of Pathology, King George's Medical University, Lucknow, for her continuous and inspiring guidance, undeterred encouragement and personal attention which cleared my

doubts and guided me in the right path. I deem myself fortunate to have worked under her guidance.

I also wish to express my regards and gratitude to esteemed teacher Dr.Mili Jain, Department of Pathology, King George's Medical University, Lucknow, or her meticulous guidance, constant encouragement and invaluable advices which helped me complete this work. Without her navigation, completion of this work would have been impossible.

I wish to thank my Colleagues, as well as my seniors and juniors who provided constant cooperation during the course of this study.I am thankful to all the technical staff of the lymphoma-leukaemia lab for all their help.

I will be failing in my obligation if I do not thank my parents, **Dr. A.S.Chauhan** and **Dr. Amita Singh**, my sister, brother and all other family members and especially my husband **Dr. Sachin Sharma**, for their all-round support and encouragement in the most demanding circumstances.

I give my special thanks to the Arun Photostat for statistical analysis as well as setting and binding of my thesis.

Last but not the least, I wish to express my gratitude and sentiments towards all those who have in some way or the other helped and guided me in completing this academic exercise and effort.

Dr. Shruti Chauhan

LIST OF ABBREVIATIONS

AML	**Acute myelogenous leukemia**
APML	**Acute promyelocytic leukemia**
BFA	**Buffered formal acetone**
CD	**Cluster of Differentiation**
EDTA	**Ethylenediaminetetraacetic acid**
FAB	**French-American-British classification**
MPO	**Myeloperoxidase**
WHO	**World Health Organisation**
AML-M0	**Acute Myeloid Leukemia with minimal differentiation**
AML-M1	**Acute Myeloid Leukemia without maturation**
AML-M2	**Acute Myeloid Leukemia with maturation**
AML-M3	**Acute Promyelocytic Leukemia**
AML-M4	**Acute MyeloMonocytic Leukemia**
AML-M5	**Acute Monocytic Leukemia**
AML-M6	**Acute Erythroid Leukemia**
AML-M7	**Acute Megakaryoblastic Leukemia**

TABLE OF CONTENTS

INTRODUCTION

Acute myeloid leukemia (AML) is a haematopoeitic neoplasm characterized by an increase in the number of myeloid cells in the marrow and an arrest in their maturation, frequently resulting in hematopoietic insufficiency (granulocytopenia, thrombocytopenia, or anemia), with or without leukocytosis1.

Malignancies of the hematopoietic system include leukemias, lymphomas, plasma cell dyscrasias, myeloproliferative neoplasms and histiocytic tumors. Leukemia was first discovered in the microscopic work of Alfred Francois Donne in 18392. The term laeukemia was coined by Virchow in 1845 3 which was characterized by too many white blood cells, thereby the term 'white blood' or leukemia.

Leukemia is broadly divided into acute and chronic. Acute leukemias are defined as hematopoietic neoplasms with more than 20% blasts in the peripheral blood or bone marrow. These are classified into myeloid and lymphoid neoplasms according to the cell of origin as they differ considerably in response to treatment therapy and course.

Acute myeloid leukemia is a heterogenous disease clinically, morphologically, genetically and differs in course and prognosis. With the recent advances made, classifying it into homogenous subtypes allows us to diagnose it, distinguish the prognostic parameters and thereby refine the treatment strategies.

The diagnosis of acute leukemia traditionally has been based on the evaluation of peripheral counts, bone marrow morphology and cytochemical staining. With the introduction and later modification of the French-American-British (FAB) classification system, based upon morphologic and cytochemical characteristics, standardization of criteria for the subclassification of myeloid and lymphoid leukemias was done.However, it is still insufficient for the classification of many leukemias, as morphologic characteristic may overlap and cytochemistry may be negative or equivocal.(4) In most cases of poorly differentiated leukemias, the lineage cannot be definitely diagnosed by these methods.

The classification of acute myeloid leukemia has revolutionised over the years. With the FAB classification system providing a subclassification of myeloid leukemia, the WHO classification has incorporated immunophenotyping methods, by which various antigens expressed by the leukemic cells can be assessed by flowcytometry through monoclonal antibodies to establish myeloid lineage to the blasts and confirm the morphological diagnosis. Thus it can be used for rendering specific treatment and predicting the outcome of different types of leukemia5.

The 4th edition of the WHO classification (2008) incorporates new information that has emerged from scientific and clinical studies in the interval since the publication of the 3rd edition in 2001, and includes new criteria for the recognition of some previously described neoplasms as well as adds entities defined principally by genetic features-that have only recently been characterized.

Leukemic cells exhibit specific antigens that correspond to the basic myeloid and lymphoid lineages and are identified by the presence of monoclonal antibodies. Flowcytometry is a technique by which such antigens can be identified. Flowcytometry, aided with appropriate antigenic markers makes possible the investigation of leukemia, differentiation and subtyping. Thecluster of differentiation (CD) is a system that involves groups of monoclonal antibodies for the identification and recognition of specific cell surface molecules present on leukocytes. The CD nomenclature was established in the 1st International Workshop and Conference on Human Leukocyte Differentiation Antigens (HLDA)7.

Studies demonstrated that when CD45 is combined with side scatter in a flowcytometer, blasts could be separated according to their lineages based on cytoplasmic complexity, the bone marrow sample is readily segregated into its cellular constituents forming clusters of cellular events counted on flowcytometric scatterogram. This enabled successful characterization of antigens on leukemic blasts using labelled antibodies8. The CD45/SSC gating procedure improved phenotypic determination of the blast cells in three ways: (1) by discriminating between leukemic blast cells and residual normal cells; (2) by excluding normal cells from the phenotypic analysis of leukemic blast cells; and (3) by identifying blast cell

heterogeneity in many cases of leukemia on the basis of different CD45 display 9.

Presently flowcytometry can be applied for diagnosing acute undifferentiated leukemia, distinguishing poorly differentiated case of acute lymphoblastic leukemia (ALL) from acute myeloid leukemia (AML), for immunological classification of ALL, to distinguish ALL from malignant lymphoma, to diagnose acute megakaryocytic leukemia, to diagnose biphenotypic acute leukemia and also for minimal residual disease detection following chemotherapy.

With the advent of sophisticated flowcytometric instruments, immunophenotyping analysis of bone marrow or peripheral blood cells has become a standard tool in the assessment of patients with leukemia and is typically used in conjunction with clinical, morphological features and cytochemical staining methods for the diagnosis of acute myeloid leukemia10.

In this study we will assess the comparison between morphological, cytochemical and flowcytometric diagnosis of acute myeloid neoplasms, thereby, arriving at a definitive diagnosis or a narrow differential.

AIMS AND OBJECTIVES

GENERAL OBJECTIVES: The aim of this study is to characterise the clinical, morphological and cytochemical features of Acute Myeloid Leukemia and correlate them with the diagnosis provided by the immunophenotyping method.

SPECIFIC OBJECTIVES:

1) Evaluating the morphological features for diagnosing and classifying Acute Myeloid Leukemia.

2) Assessment of the cytochemical staining methods (as and when required) for diagnosing and classifying Acute Myeloid Leukemia.

3) To evaluate the usefulness of the information provided by flowcytometric immunophenotyping for establishing the diagnosis and classification of Acute Myeloid Leukemia.

To Compare the immunophenotype obtained in each case with its corresponding FAB morphological diagnosis and to determine correspondence between FAB myeloid subtypes and the immunophenotyping pattern.

REVIEW OF LITERATURE

Acute myeloid leukemia is a complex and heterogenous disease characterized by an arrest of normal hematopoetic maturation produced by dysregulated clonal expansion of karyotypically distinct neoplastic cells. The requisite blast percentage is 20% or more myeloblasts and/ or monoblasts /promonocytes /or megakaryoblasts in the peripheral blood or bone marrow 11.

Acute myeloid leukemia is the most common type of leukemia in adults (about 25% of all leukemia) in the western world. Worldwide the incidence is approximately 3.7 per 1,00,000 population per year. The median age of diagnosis is 65 years. In pediatric age, Acute myeloid leukemia comprises of only 15 -20 % cases of leukemia with a peak incidence at 3-4 years of age 12.

Patient typically presents with anemia (nonspecific fatigue), neutropenia (fever due to infections) and/ or thrombocytopenia (haemorrhagic symptoms including petechiae, epistaxis, easy bruising, etc) resulting from impaired hematopoeisis due to replacement or suppression of bone marrow elements by malignant blasts. The most common complaint is nonspecific fatigue. Fever is found in 15 to 20% patients, weight loss in upto 50% patients and bone pain in upto 20% patients. Organomegaly and adenopathy is seen in 50% cases. Disseminated intravascular coagulation is found especially in Acute promyelocytic leukemia. Apart from this, leukemia cutis is found in upto 13% of patients (especially in those with a monocytic component). Myeloid sarcoma is seen in upto 2 to 14% patients. These clinical features can also provide clue to the diagnosis 13.

The white cells, 'the globuli albicantes', were first noted by Joseph Lieutaud (1703–80) (Lieutaud, 1749), the French anatomist. Some 20 years before, William Hewson (1739–74), anatomist, published his major work on the lymphatic system and first described the lymphocyte (Hewson, 1774). Early in the nineteenth century, a number of cases of patients with peculiar alterations of the blood was published. It was in 1811, that P. Cullen described

a case of splenitis acutus with unexplainable milky blood. Later in 1825, A. Velpeau defined the leukemia associated symptoms, and observed pus in the blood vessels. In 1844, Donne discovered that more than half of the cells were mucous globules or white cells in a patient's blood. He detected a maturation arrest of the white blood cells thereby contributing towards the pathogenesis of leukemia.

Leukemia was not officially diagnosed until 1845, when John Hughes Benett diagnosed it in Edinburgh (Greaves, 2000). J. Bennett named the disease as leucocythemia on the basis of microscopic accumulation of purulent leucocytes. Other European physicians in the 19th century observed that their patients had abnormally high levels of white blood cells, and they called the disease "weisses blut," meaning "white blood". Contemporarily, Rudolf Virchow observed a reversed white and red blood cell ratio with an abnormally large number of white blood cells in a blood sample from a patient. He called the condition Leukämie in German, which he formed from the two Greek words, leukos meaning "white" and haima meaning "blood" in 1847. In 1946, H. Fuller performed the first microscopic diagnosis of a leukemic patient during life 15. Around ten years after Virchow's findings, pathologist Neumann found that the bone marrow in a deceased leukemia patient had "dirty green-yellow" color as opposed to the normal red. He thus concluded that a bone marrow abnormality was responsible for the leukemic blood picture 16.

In 1900 Naegeli described the myeloblast and the identification of this cell as a member of the myeloid series, made it possible to recognize many cases of leukaemias. Later Reschad and Schilling-Torgau (1913) described what they thought was a case of monocytic leukaemia, and their description suggested that there might be an additional form of leukaemia distinct from those recognised at that time. In subsequent years leukaemia without leukocytosis ("aleukaemic leukaemia") was recognized. The leukemic myeloblast, monoblast and erythroblast were identified. The myeloblast is a unipotent stem cell, which will differentiate into one of the effectors of the granulocyte series.

Subsequently, it was discovered that few acute leukemias were marked only by abnormal leukocytes in the blood without

accompanying leukocytosis. By the dawn of 20th century, the leukemias were viewed as a group of diseases contrary to a single disease leading to the development of various approaches in the classification of leukemias 18.

It was in 1891, when Ehrlich had introduced cytochemical methods of staining for the morphological differentiation of leukocytes and identification of leukemia cell types 17. Subsequently, different studies were carried out to identify the lineage of white blood cells by their cytoplasmic contents using specific stains.

Myeloperoxidase (MPO) is a peroxidase enzyme that in humans is encoded by the MPO gene on chromosome 17. MPO is most abundantly expressed in neutrophil granulocytes. 19Demonstration of leukocyte myeloperoxidase allowed sensitive identification of cells of granulocytic series.

In 1965, benzidine dihydrochloride was used for localizing intracellular peroxidase activity, as an indicator compound present in a stable, reusable staining solution by Leonard S. Kaplow20. In 1966, Graham et al.21 studied that 3, 3′ Diaminobenzidine produced results comparable to benzidine hydrochloride for localization of peroxidases in the cells along with being a safer alternative.

In acute leukaemias, the cell identification is often problematic as there is a marked similarity between earlier precursors of different cell series. The presence of cytochemically stainable structures, in earlier precursors, indicate specific cellular differentiation which the precursor cells are undergoing, thus simplifying the identification. These developments in leukocyte cytochemistry further aided in identifying and classifying leukemias 23.

In 1978, Neelam R Batra et al studied cytochemical staining in cases of leukemia and observed that in cases where the diagnosis was disputable on the basis of morphology using Romanowsky stained smears, cytochemistry helped in differentiation of blast cells 24.Also, in 30% of cases that were previously diagnosed as lymphoblastic leukaemia on Romanowsky stains alone, cytochemistry revealed a myeloid pattern with the use of Peroxidase.

In 1984, work was done by Jacob S Hanker on cytochemical staining of AML.

In 1970, another milestone was added, it was first confirmed that some patients could be cured of leukemia, and by the 1980s and 1990s the cure rates for leukemia were around 70% (Pui, 2003)12.

The French-American-British (FAB) classification of acute leukemia was proposed in 1976 to standardize the morphologic and cytochemical classification of acute leukemia25, a group of seven French, American and British haematologists conducted a study on 200 patients of acute leukaemia, and three major hematological malignancies- acute lymphoblastic leukemia (ALL), acute myeloid leukemia(AML) and myelodysplastic syndrome (MDS) were categorised 26 .

Romanowsky morphology and cytochemical stains were used, which included myeloperoxidase (MPO) and Sudan Black B (SBB) for granulocytic differentiation and nonspecific esterase (NSE) for monocytic differentiation. Initially six subtypes were identified: six myeloid (Ml through M6) and three lymphoid (L1through L3) but a further megakaryoblastic subtype M7 was added by introducing revisions in 1981 and 1985. In the majority of cases of acute myeloid leukemia, a variable proportion of the leukemic cells (blasts) are reactive for myeloperoxidase and Sudan black B, whereas the stains are uniformly negative in ALL. However studies demonstrated that cytochemical stainslack diagnostic sensitivity in over 20% of acute leukemias.

M1: Predominance of myeloblasts with <10% granulocytic differentiation

M2: >30% myeloblasts with >10% differentiating granulocytes

M3: Hypergranular promyelocytes with numerous auer rods NSE<20%

M4: Monocytic cells >20% but <80% NSE positivity

M5: Monocytic cells with >80% NSE positivity a)monoblastic b) monocytic

M6: >50% erythroblasts with >30% myeloblasts

M7: Megakaryoblastic (M7 was added later in 1985)

Historical classification systems relied on morphological and cytochemical features, but currently immunophenotyping are being incorporated to diagnose acute leukemia. Infact, the fourth

edition of WHO classification of hematolymphoid neoplasm has moved beyond morphology (FAB Classification) to integrate clinical information, morphology, imunophenotyping by flowcytometry or immunohistochemistry, cytogenetics and molecular data.27.

The first impedance-based flow cytometry device, using the Coulter principle, was disclosed in U.S. Patent 2,656,508 in 1953, to Wallace H. Coulter. This laid the foundation for the first impedance-based flow cytometry device developed by Mack Fulwyler as the forerunner to today's flow cytometers - particularly the cell sorter28. The first fluorescence-based flow cytometry device was developed in 1968 by Wolfgang Göhde29.

In 1972, Dr. Len Herzenberg's group at Stanford University designed and patented a fluorescence activated cell sorter (FACS). In 1974, Becton Dickinson, Inc. introduced the first commercial flow cytometer, which was called the FACS-1, followed in 1977 by the System 50 Cytofluorograph, developed by Ortho Diagnostics, Inc.

By the end of the 1980's, Becton Dickinson's FACScan and Coulter's EPICS Profile flow cytometers had become industry-standard commercial cell analyzers. Then, in 1994, Cytomation introduced the MoFlo® cytometer, a commercial version of the high-speed cell sorter developed by Dr. Ger van den Engh and his colleagues. Today, new applications for flow cytometers require specialized features and functions. The potential for use in research continues to grow, ensuring the future of the science of flow cytometry in both the academic and commercial research communities30.

Subsequently, studies were done for analysing the antigenic expression on leukocytes. In 1983, James D. Griffin et al.31 studied about the MY9 antigen expressed by blasts, promyelocytes and myelocytes in the bone marrow, and by monocytes in the peripheral blood and negative for erythrocytes, lymphocytes and platelets. Also, the leukemic stem cells (leukemic colony-forming cells, L-CFC) came out to be MY9 positive. Contrarily, MY9 was not detected on lymphocytic leukemias. Thus, they concluded that Anti-MY9 may be valuable for the identification of myeloid-lineage leukemias, and hence, for a highly reproducible classification system that can provide additional information about the leukemic cells along with

morphological analysis.

Adding to the work done in the field of cytochemical diagnosis of AML, in 1985, Masao Tomonaga et al conducted a study on cytochemical characteristics of cases of acute promyelocytic leukemia.32 They defined two major categories based on cellular differentiation, one including the pure neutrophilic type (16/25 cases) with strong myeloperoxidase (MPO) and naphthol AS-D chloroacetate esterase, but lacking the monocytic enzyme, sodium fluoride (NaF) -sensitive alpha-naphthyl butyrate esterase and the second category including the mixed neutrophilic/monocytoid type (7/25 cases) with strong esterase as well as strong MPO positivity.

Later, Davey et al studied the morphologic and cytochemical characteristics of acute promyelocytic leukemia in 2006. Diagnosis of promyelocytic leukemia is especially critical as we know that the cure rate exceeds 75% in this subtype since the introduction of retinoic acid-containing regimens71.

A revision was done by the French–American–British Cooperative Group (FAB) in 1985, and they used the 30% bone marrow blast cells as the cut off limit. Also, they attempted to define type I and II myelocytes for defining APML as the neoplastic cells in this subtype were promyelocytes (myeloblasts II) and thus it did not fulfil the criteria of 30% blasts.26,34

A further revision was proposed in 1991 to include leukemia types (minimally differentiated acute myeloid leukemia categorized as AML-M0) that could be accurately identified only with the addition of immunophenotyping or electron microscopic studies.35

Peter B Neame in 1986 attempted a combined FAB-Immunological classification of AML. A panel of commercially available monoclonal antibodies and five heteroantisera were used to distinguish and subtype 138 cases of acute leukemia (AL). The immunophenotype was compared with the French- American-British (FAB) classification obtained in the cases. The immunophenotype discriminated acute myelogenous leukemia (AML) from acute lymphoblastic leukemia (ALL) and also recognized the cases which were not distinguished by cytochemistry (22% cases), mixed lineage phenotypes (13% cases), and cases with separate populations of lymphoblasts and myeloblasts (one case). Correspondence of the

immunophenotype to the FAB subtypes M1, M2, M4, and M5 was possible in greater than 80% of cases, using the immunologic panel and derived criteria to subtype AML. A combined classification of the immunophenotype and FAB morphology/cytochemistry was devised for AML subtyping.

The revised FAB classification36 is summarized as follows:

M0 AML with no Romanowsky or cytochemical evidence of differentiation

M1 Myeloblastic leukemia with little maturation

M2 Myeloblastic leukemia with maturation

M3 Acute promyelocytic leukemia (APL)

M3h APL, hypergranular variant

M3v APL, microgranular variant

M4 Acute myelomonocytic leukemia (AMML)

M4eo AMML with dysplastic marrow eosinophils

M5 Acute monoblastic leukemia (AMoL)

M5a AMoL, poorly differentiated

M5b AMoL, differentiated

M6 "Erythroleukemia"

M6a AML with erythroid dysplasia

M6b Erythroleukemia

M7 Acute megakaryoblastic leukemia (AMkL)

In 1986, George P. Browman et al.37 attempted a study for the intraobserver and interobserver reproducibility of the FAB classification. Intraobserver reproducibility was assessed for Wright-stained preparations that were examined independently on two consecutive occasions, 2 weeks apart. A third reading was performed with Wright stain and cytochemical data, and the fourth reading was done with the aid of immunophenotype data. Concordance between observers was found to be 63% and 72% for each of two separate readings using Wright-stained preparations, that rose to 89% when cytochemistry was added and further to 99% when immunophenotyping was included. Discordance between observers occurred on nine occasions when Wright-stained

preparations only were used, four times when cytochemistry was used while it did not occur with immunophenotyping. The study suggests that immunophenotyping, as an aid to morphological assessment of acute leukemia, contributes enormously to concordance between observers.

Similar study was also conducted by Head et al.(40) and they reported a lower concordance rate as their study was based only on morphological features.

The National Cancer Institute (NCI) in 1990 (B D Cheson et al) (59) tried to develop a set of standardized diagnostic and response criteria for acute myeloid leukemia (AML) clinical trials. They reported four important subtypes of AML not defined in the FAB classification: undifferentiated acute leukemia, MO (AML lacking definitive myeloid differentiation by morphology or conventional cytochemistry but with ultrastructural or immunophenotypic evidence for AML), mixed lineage leukemia, and hypocellular AML.

Adriano Venditti et al in 1994 described 19 cases of AML-MO categorized among 200 consecutive AML cases. Cytochemistry (MPO, SBB, αANAE, αNBE, NASDCAE, AP, PAS) was negative in 14/19 cases, with five cases expressing a very faint cytoplasmic positivity for αNBE and αANAE which was sodium fluoride resistant. In these five cases, CD14 did not favour myelomonocytic differentiation. All the cases were anti-MPO positive and were positive for at least one myeloid antigen, Tdt was expressed in nine cases and CD7 in six cases. All cases but one were positive for CD34. Later on, three out of five patients with faint αNBE/αANAE positivity, relapsed as typical M4 (one case) or M5a (two cases) 63.

In 1997, F Lacombe et al.39 studied a flowcytometry method into the routine investigation of whole bone marrow samples following red blood cell lysis on the basis of a primary CD45/ side scatter (SSC) gating procedure. Blast cells were first identified byCD45/SSC gating in 74 cases of acute myeloid leukemia (AML) and the results were compared to a conventional FSC/SSC gating procedure and to MGG-staining smears. They described that percentages of blast cells as defined by the morphological analysis better correlated with the values determined by CD45/SSC gating (r

5 0.94) than with the blast cell counts recorded with FSC/SSC gating (r 5 0.76). Thus, they inferred that CD45/SSC gating procedure improved phenotypic determination of the blast cells, enabled an efficient discrimination between the various cell lineages and analysis of leukemic blasts present in low proportions.

Cesare Guglielmi et al in 199856 compared the immunophenotype of adult and childhood acute promyelocytic leukaemia with morphology, type of PML gene breakpoint and clinical outcome in 196 molecularly confirmed APLs (63 children and 133 adults) in Italy. They arrived at a conclusion that APL has immunophenotype characterized by frequent expression of CD13, CD33 and CD9 and rare expression of HLA-DR, CD10, CD7 and CD11b. However, CD2 was seen in 28%, CD34 in 23% and CD19 in 11% of cases. Thus, immunophenotype plays a role in the determination of the biological and clinical heterogeneity of childhood and adult APL.

Immunophenotyping is useful for predicting the biologic and clinical behavior of APL57.

A study in 1998 conducted by Samir A. Kheiri et al.42 retrospectively analysed 93 cases of acute leukemias and diagnosed them as AML, ALL, biphenotypic and non diagnostic categories. AML and ALL showed high lineage concordance by both modalities. But, when nondiagnostic or biphenotypic diagnoses made by either methodology were included, complete concordance was seen in only 77.4% of cases. It was also concluded that diagnostic sensitivity was lacking in 20% of acute leukemias by cytochemical stains.

FAB was revised to include leukemia typesthat could only be accurately identified with the addition of immunophenotyping orelectron microscopic studies (Bennett et al., 1991) 35.

Although the FAB classification did not recognizethe significance of myelodysplastic changes in acute myeloid leukemias or cytogeneticabnormalities in leukemia types, this system rendered very clear criteria for classification. Also, some distinct leukemia subtypes, particularly acute promyelocytic leukemia and acute myeloid leukemia with abnormal eosinophils, correlated well with the specific cytogenetic aberrations and had unique clinical features, and those remain in recently proposed classification systems.

It was in 2002, WHO classification was introduced by World Health Organization (WHO), in association with the Society for Hematopathology and the European Association of Hematopathology43. The classification schemes by the World Health Organization (WHO) require the additional evaluation of the leukemic blasts by molecular analysis and flow cytometry (Harris NL 1997 & Brunangelo Falini 2010, Sachdeva et al 2006). The results of these 4 methods of evaluation (i.e, morphology, staining, molecular analysis, flow cytometry) also categorized the subtypes of acute leukemia apart from differentiateing ALL from AML,

Later, World Health Organization Classification of Acute Myelogenous Leukemia (2008) was revised which is shown in Annexure 2.

The two most significant differences between the FAB and the WHO classifications are:

(a) A lower blast threshold for the diagnosis of AML: The WHO defines AML when the blast percentage reaches 20% in the bone marrow.

(b) Patients with recurring clonal cytogenetic abnormalities should be considered to have AML regardless of the blast percentage (8;21)(q22;q22), t(16;16)(p13;q22), inv(16)(p13;q22), or t(15;17)(q22;q12) (Arber et al 2008, Weinberg et al 2009).

Since 1998, AML has been divided into three cytogenetic risk groups that help define treatment (see Annexure 4)

It was observed that further subdivision of myeloid neoplasms into acute promyelocytic (M3) and other FAB groups was required that with the developing new treatment regimes for acute leukemia. 47

In 2004, S.B. Kresno et al.48 analysed morphological, cytochemical and immunophenotyping data from 225 leukemic patients over a period of 7 years. Although morphological findings and cytochemistry still are needed for preliminary diagnosis of acute leukemia, flowcytometric analysis of antigenic expression of tumor cells helps in further characterization. Also expression of non-lineage markers was demonstrated.

Ansari et al (2003) retrospectively analysed Haematologic and immunophenotypic profile of acute myeloid leukemia of 260 cases of AML diagnosed between 1998 and 2000 diagnosed at Tata Memorial Hospital. Seventy-six percent of our cases were adults. The age of the patients ranged from one year to 78 years with a median age of 27.2 years. 187 males and 73 females were seen. The commonest FAB subtype, in both children and adults, was AML-M2. The highest WBC counts was seen in AML-M1 and the lowest in AML-M3 (10-97 x 10(9)/L, mean 53.8 x 10(9)/L). The mean values and range for hemoglobin was 6.8 gm/l (1.8 gm/l to 9.2 gm/l), peripheral blood blasts 41.4% (5 to 77%), platelet count 63.3 x 10(9)/L (32-83 x 10(9)/L), and bone marrow blasts 57.6% (34-96%). CD13 and CD33 were the most useful markers in the diagnosis of AML. Myeloperoxidase positivity was highest in the M1, M2 and M3 subtypes. CD14 and CD36 were most often seen in monocytic (38%) and myelomonocytic (44%) leukemias. CD7 expression was the commonest (11%) aberrant lymphoid marker. AML accounted for 39.8% of all acute leukemias at this institution. The most common subtype was AML-M2. CD13 and CD33 were the most diagnostic myeloid markers.46

Immunophenotyping was studied in cases of acute leukemias along with expression of aberrant markers in leukemic cells with the related cytogenetic abnormalities.

Y Xu et al in 2006 61 diagnosed and retrospectively analysed a total of 126 cases of de novo AML with 4-color flow cytometry at their institution from 1998 to 2004. By FAB criteria, 54 exhibited morphologic and cytochemical features of monocytic AML, including 31 cases of M4 and 23 cases of M5 (15 monoblastic and eight monocytic). Those 54 cases were classified by WHO criteria as AML with t(8;21) (3), inv(16)/t(16;16) (7), or 11q23 (11); AML with multilineage dysplasia (3); or AML NOC (30). Of the 30 AML NOC cases, 17 were classified as AMML and 13 as AMoL.

In 2006, Misbah Qadir et al.50 retrospectively analysed 646 cases of acute leukemia. Of these, lineage was established in 612 cases by morphology and cytochemistry alone. The rest 34 cases were diagnosed only on the basis of immunophenotyping of which 26, 5, and 3 were classified as myeloid, undifferentiated,

and lymphoid leukemias respectively. In about 8 cases, the immunophenotyping changed the diagnosis as earlier made by morphology and cytochemistry. Thus, immunophenotyping is as an essential modality that should be included in routine practices.

Ihsan M. Osman et al conducted a study to characterize immunophenotypic patterns of AML in 106 sudanese cases of AML during the period mid-2010 to mid2011. The following antigens: CD45, HLA-DR, CD34, CD117, CD13, CD33, CD19, CD7, cytoplasmic markers (CD3, CD79a, MPO), CD11c, CD14, CD64, CD42a, CD41 and CD61 were used. CD13 and CD33 had positivity of 51.5% and 49.8% respectively in all AML subtypes collectively. CD33 was found to have higher positivity among AML-M4 and AML-M5 with mean positivity of 75.9% and 76.6% respectively. Apparent expression of CD7 and CD19 were expressed in 45.1% and 13.6% of all cases respectively. CD7 was mostly expressed in AML-M2 and AML-M3 (75%) and least in AML-M5, while CD19 was only expressed in cases of AML-M0 and AML-M7.55

In 2008, A. Plesa et al.51 studied the use of flowcytometry for distinguishing between the certain subgroups of AML (M0-M7) as formulated by FAB classification, on the basis of CD34, CD36 and HLA-DR expression by leukemic cells. Their aim was to differentiate myelo-monocytic progenitor cell (MMPC) into either monoblasts or myeloblasts. In both these cases, expression of CD34 specific for MMPC decreases and CD36 expression was found to be increased while it was not the case for myeloblasts. Thus, they were able to divide all cases in groups as M0/M1, M2/M3 and M4/M5.

A Study was done by Jine Zheng et al (in 2008) to elucidate the immunophenotype of acute myeloid leukemia (AML) and characterize the correlation among morphological, immunological, cytogenetic, and clinical features. Bone marrow immunophenotypes of 180 AML was studied and was found that CD34, CD2, CD14, CD19, CD56, and HLA-DR correlated with FAB subtypes. Thus concluding that immunophenotypic analysis was useful for AML diagnosis and classification. At the same time, the data also suggested that the karyotype abnormalities and clinical features were tightly linked with abnormal antigen expression characteristics in AML patients 45.

In 2010, Mukda et al.52 conducted a study on 114 cases and divided them as AML and ALL on the basis of morphology. Concordance was seen in 89.6% and 82.35% cases after cytochemical staining for MPO and PAS, respectively. Flowcytometry further confirmed the morphological diagnosis. Also they reported a change in diagnosis in one case where morphology suggested lymphoid lineage but on flowcytometry, myeloid markers (CD13, CD33) were positive.

Dalia A Salem in 2011 evaluated the diagnostic usefulness of commonly used immune-markers for immunophenotyping of Acute leukemia and to define the best immune-markers to be used for proper diagnosis and classification. Among the 164 patients studied, 68.9% were classified as acute myeloblastic leukemia (AML) while 31.1% classified as acute lymphoblastic leukemia (ALL). The commonest FAB subtype in AML group was AML-M4/5 (34.5%) which may differ from most published data. It was also inferred that combined use of HLADR and CD34 was more helpful in differentiating APL from non-APL AML than either of these antigens alone. Flowcytometry was found to be especially useful in the identification of AML-M0 and differentiation of APL from non-APL AML. 54.

In 2013, Sushma Belurkar et al.44 analysed 50 cases of acute leukemia and found complete concordance in the data on morphological, cytochemical and immunophenotyping grounds in 58% of the cases where flowcytometry confirmed the diagnosis. In 22% and 4% of the cases where there was partial and complete discordance in morphological and cytochemical pictures respectively, flowcytometry helped in identifying the correct subtype. The rest 16% of the cases were those where flowcytometry was used for the final diagnosis as definitive diagnosis could not be made by morphology and cytochemistry.

Eddy Supriyadi et al in 2011 concluded in their study that immunophenotyping offers a better classification of the hematopoietic lineage of malignant cells as compared to morphology and may improve the diagnosis of childhood leukemia. 541 suspected leukemia patients were analysed over a 4-year period. Immunophenotyping allowed classification into AML and ALL.

Concordance with morphology was very good (κ=0.82) using the three-color method with a panel of 15 monoclonal antibodies. Nine out of 239 ALL cases diagnosed morphologically were finally labelled as AML, and 12/79 morphological AML came out to be ALL. Immunophenotyping is particularly important for assessment of prognosis inchildhood leukemia even in low income countries such as Indonesia 60.

Flowcytometry plays a well established role in diagnosis of acute myeloid leukemia principally for blast enumeration, lineage assignment, identification of immunophenotyping and post therapeutic disease monitoring. Multiparametric flowcytometry provides rapid and detailed determination of antigenic expression profiles in acute leukemias which in conjunction with morphological assessment, often suggests a definitive or narrow differential 27,62 It is used to differentiate AML from ALL by establishing myeloid lineage to the blasts. It is used to confirm the morphological findings in AML with telltale features eg. Auer rods, hypergranular promyelocytes as well as, for rest of the cases i.e. AML without maturation, AML MO64, blasts without auer rods and hypogranular promyelocytes, acute monoblastic leukemia, acute erythroleukemia and acute basophilic leukemia.Acute megakaryoblastic leukemia can be confused with ALL –L1 and AML M0. It is difficult to be diagnosed alone, and needs immunophenotyping as a gold standard 65.

Immunophenotyping is of immense use in diagnosing AML MO 66. Flowcytometry rapidly identifies acute promyelocytic leukemia with high specificity independent of underlying cytogenetic abnormalities 67.

Most cases of acute leukemia can be classified as myeloid or lymphoid on the basis of morphology and cytochemistry. There are, however, some cases that remain difficult to classify due to the co-expression of several myeloid and lymphoid antigens in the same cells. These have been designated as biphenotypic or mixed lineage acute leukemia68. Immunophenotyping can be helpful in such cases by identifying myeloid associated antigenic expression of ALL (MY + ALL) or lymphoid associated antigenic expression of AML (LY +AML) 27.

Mehdi Jaredi et al79 in 2014 did a study to evaluate the incidence of aberrant phenotypes and possible prognostic value in peripheral and bone marrow blood mononuclear cells of Iranian patients with AML. Out of the 56 cases, 32 (57.1%) showed aberrant expression. CD7 was positive in 72.7% of cases in M1 and 28.5% in M2 but M3 and M4 cases lacked this marker. CD2 was seen in 58.35 of M1 cases, 21.40% of M2 cases, 33.3 of M3 and 20% of M5; but M4 patients lacked this marker.

Immunophenotyping is useful in the early detection of Minimal residual disease. It can be used for the valid quantification of minimal residual disease both during aplasia following induction therapy and after achievement of complete remission. It may thus represent a tool providing important prognostic information in patients with acute myeloid leukemia69,70.

Characteristic antigen expression patterns have been found to be associated in acute myeloid leukemia with recurrent cytogenetic abnormalities. These may help in planning directed FISH or PCR studies.

Immunophenotyping may also indicate groups with poor prognosis, such as those with CD7 or CD34 positivity.

Infact, flowcytometry has contributed a lot to the reproducibility of the FAB classification in acute leukemia. The remarkable consistency of the immunophenotyping results (in one of the studies done) suggests that surface marker analysis of acute leukemia should be an important element in the classification of this disease37. Hence, a multifaceted approach should be applied by integrating clinical, morphological features with the flowcytometric immunophenotyping findings for the diagnosis and classification of acute myeloid leukemia and not just relying solely on morphological diagnosis72.

MATERIALS AND METHODS

The study was conducted at the Department of Pathology, King George Medical University, Lucknow.

Study duration: One year (2014-2015)

Study design: Prospectiveand retrospectivedescriptive study

DATA COLLECTION:

Newly diagnosed cases of acute myeloid leukemia were asked for personal identification details, disease symptoms, any presenting sign and symptoms of cytopenia like fatigue, weakness, dizziness, bleeding tendencies and infections and data about investigations already done.

Inclusion criteria for this study:

- Patients newly diagnosed with acute leukemia
- Patients who gave written informed consent for undergoing bone marrow aspiration.

Exclusion criteria for this study:

- Patients on chemotherapy
- Patients with coagulation disorder

PLAN OF STUDY

- Detailed clinical history for presenting symptoms was taken.
- We examined the patients for presence of features of anemia, any bleeding manifestations, gum hypertrophy, hepatosplenomegaly and lymphadenopathy.
- Following investigations were done in each patient
- Hemoglobin estimation, total and differential leukocyte counts and platelet counts
- General blood picture and blast identification and percentage
- Bone marrow aspirate examination for the presence and percentage of blasts.
- Cytochemical staining for Myeloperoxidase.

• Flowcytometric analysis of marrow aspirate for markers specific for myeloid as well as the aberrant markers.

• Cytogenetic study whenever permissible.

SAMPLE COLLECTION:

• 2 ml of peripheral blood sample was collected in EDTA vaccutainer for hemoglobin estimation and smears were prepared for total and differential leukocyte counts, platelet counts and general blood picture examination with blast counts.

• Bone marrow aspiration was done in all patients under local anesthesia using approximately 2ml of 2% lignocaine solution.

• Collected aspirate was used to prepare smears and the remaining sample was transferred to EDTA vaccutainer and refrigerated at 8˚C for flowcytometric analysis to be done within the next 24 hours.

EXPERIMENTAL DETAILS:

MORPHOLOGICAL EXAMINATION:

Leishman stain:

• **PRINCIPLE:** Leishman stain is a type of Romanowsky stain which distinguishes between the cellular components by subtle differences in the shades of staining. The dye has two components: Azure B and Eosin Y. Azure B reacts with the anionic molecules thereby staining the nucleic acid, proteins of cell nuclei, neutrophilic granules and basophilic granules. Eosin Y bind with the cationic molecules and hence stains the hemoglobin molecule and granules of eosinophils.

• **REAGENT USED AND STAIN PREPARATION:**

Prepared powdered Leishman stain (Thomas Baker) was used.

2.0 gm of powdered dye was dissolved in 1000ml methanol

This was warmed to 50˚C for 15 minutes with occasional shaking.

The solution was allowed to cool and kept for few days to allow maturation.

•**METHOD:** Air dried blood and bone marrow aspirate smears were placed on the staining rack and flooded with Leishman stain. After 2 minutes, the slides were flooded with double the

amount of water and left for 5-7 minutes. The slides were then washed with buffered water, air dried and examined.

• **INTERPRETATION:** The chromatin stains purple and nucleoli stain light blue. Cytoplasmic and granular staining is variable depending on the type of cells. Cytoplasm of granulocytic precursors stain pinkish. The granules of neutrophils and precursors stain red to purple. Eosinophils granules stain red to orange. Basophil granules stain purple black. Cytoplasm of lymphoid cells take bluish stain. Erythroid precursors show dark blue to pink cytoplasm in early to late normoblasts. RBCs stain pink in colour.

• The blasts were identified on the basis of their size, nuclear chromatin, nucleoli, cytoplasm and cytoplasmic granules,. Myeloblasts varied in size from slightly larger than mature lymphocytes to the size of monocytes or even larger, with moderate to abundant amount of cytoplasm staining dark blue to blue-grey. The nuclei were large, round to oval with finely granular chromatin and multiple nucleoli. Few azurophilic granules were occasionally present along with presence of Auer rods which stained purple.

CYTOCHEMICAL TEST:

Myeloperoxidase:

• PRINCIPLE: The enzyme myeloperoxidase (MPO) is present in the primary and secondary granules of neutrophils and its precursors, in granules of eosinophils and azurophilic granules of monocytes. It splits H2O2, which in the presence of a chromogen (3,3′diaminobenzidine as used here) gives an insoluble reaction product.

• **REAGENTS USED AND STAIN PREPARATON:**

Fixative: Buffered formal acetone (BFA)

We dissolved 20mg Na2HPO4 and 100mg KH2PO4 were in 30ml of distilled water. 45ml of acetone and 25ml of 40% formalin were added to the solution. Mixed well and stored at 4˚C. Used fresh fixative made every 4 weeks.

Buffer: Sorensen's phosphate buffer, pH 7.3

Stock solution was prepared by making 66mmol/l solution

of KH2PO4 (9.1gm/l) and Na2HPO4.2H2O (11.9gm/l). Then we mixed the above in a ratio of 19.2:80.8, thus giving the required buffer of pH 7.4

Hydrogen peroxide: H2O2, 30% w/v

Substrate: 3-3′ Diaminobenzidene (Sigma D-8001)(DAB)

Working substrate solution: Prepared by mixing 30mg of DAB in 60ml of phosphate buffer and added 120µl H2O2 and mixed well.

Counterstain: aqueous hematoxylin

- **METHOD:**

The air dried smears were fixed in cold BFA for 30 seconds. The slides were then rinsed thoroughly in gently running tap water and air dried. The smears were then placed in the working substrate solution and incubated for 10 minutes at room temperature. After the incubation period, the slides were washed in gently running tap water and counterstained in hematoxylin, rinsed, air dried and mounted for viewing.

- **INTERPRETATION:**

The presence of brown granular reaction product is considered as positive. Primitive myeloblasts are negative or show very faint positivity. Intensity of staining increases as they mature, with promyelocytes and myelocytes showing strong positivity with granules filling the cytoplasm. Auer rods were better visualized. Neutrophils show granular positivity and were considered as internal control. Eosinophils also stain strongly but their granules are larger in size as compared to neutrophils. Monocytes showed variable staining. Basophils that were rarely seen, were negative.

Early myeloblasts, lymphoblasts, lymphocytes, basophils and plasma cells are peroxidase negative. Red cells and erythroid precursors show diffuse brown cytoplasmic staining but were considered negative.

FLOWCYTOMETRY

Immunophenotyping was done by a multicolor flowcytometer. The instrument used was BD FACS Caliber (Becton Dickinson-Fluorescence Assisted Cell Sorter). We used appropriate

pairs of monoclonal antibodies conjugated to fluorescein isothiocyanate (FITC), phycoerythrin (PE) or peridinin-chlorophyl-protein (PerCP) and co-expression of different lymphoid and myeloid antigens was confirmed

- **PRINCIPLE:** The basic principle of flowcytometry relies on the passage of individual cells in a suspension through a tube at a constant velocity which is illuminated by a focused light source (laser beam) and study the light scattering pattern by detector positioned at specific angles to get information about the size and complexity of the cells. We label the cells with a specific antibody, tagged with a flourochrome, thus, we can use this principle to study various antigens present on the cells and thus define the lineage in leukemic blasts with better precision.

REAGENTS USED:

Fluorochrome labeled antibodies: Acute leukemia panel was used which included the following antibodies:

PerCP: CD45

FITC: CD2, CD7, CD10, CD20

PE: CD34, CD13, CD33, CD117, CD19, CD3, CD14

BD FACSLYSE solution: working solution was made by diluting with distilled water in the ratio of 1:10.

METHOD

Stain-Lyse-Wash method:

- The appropriate number of FACS tubes were labeled for the identification of the patient and the combination of fluorochrome conjugated monoclonal antibody.
- In each tube, 100µl of sample (bone marrow aspirate or whole blood) was pipetted.
- 20µl of antibody/antibody cocktail was added to each tube.
- The tubes were incubated in dark for 10-15 minutes.
- After the incubation time, 2ml of diluted FACSLYSE solution was added to each tube.
- The tubes were again incubated in the dark for 10-12minutes. This time period was crucial as incubation for a longer duration would degrade the cells of interest while a shorter time period

would lyse the RBCs incompletely thereby increasing the debris.

- The samples were then centrifuged at 200-300g for 3-5minutes. The supernatant was discarded, the pellet formed at the bottom of the tube was broken and the remaining cells were washed twice with sheath fluid (by adding about 2ml of sheath fluid, spinning at 200-300g for 3-5 minutes and then discarding the supernatant).

The cells were again resuspended in about 0.5ml of sheath fluid and run on a pre-caliberated flowcytometer.

DATA ANALYSIS

The statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 15.0 statistical Analysis Software. The values were represented in Number (%) and Mean±SD.

The following Statistical formulas were used:

1. Mean: To obtain the mean, the individual observations were first added together and then divided by the number of observations. The operation of adding together or summation is denoted by the sign Σ.

The individual observation is denoted by the sign X, number of observation denoted by n, and the mean by .

$$\overline{X} = \frac{\Sigma X}{\text{Number of observations } (n)}$$

2. Standard Deviation: It is denoted by the Greek letter Σ.

$$\sigma = \sqrt{\frac{\Sigma (X - \overline{X})^2}{n}}$$

3. Chi square test:

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

Where O = Observed frequency

E = Expected frequency

4. Level of significance: "p" is level of significance

p > 0.05 Not significant

p <0.05 Significant

p <0.01 Highly significant

p <0.001 Very highly significant

OBSERVATIONS AND RESULTS

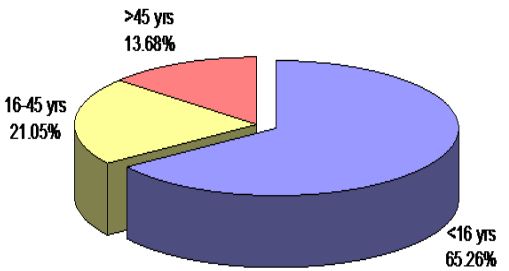
Our study evaluated the peripheral blood and bone marrow aspirate smears in acute myeloid leukemia for morphological diagnosis and compared it with their cytochemical diagnosis and immunophenotypic profile. We collected data from 95 patients, of all ages and either sex.

A. Patient characteristics:

The basic characteristics of the patients are presented in Tables 1-5 and also shown graphically alongside.

Table 1: Distribution of Study Population according to age (N=95)

Age Group	Number	Percentage
Children (<16 yrs)	62	65.26
Adults (>16 yrs)	33	34.74
Young adults (16-45 yrs)	20	21.05
Old adults (>45 yrs)	13	13.68

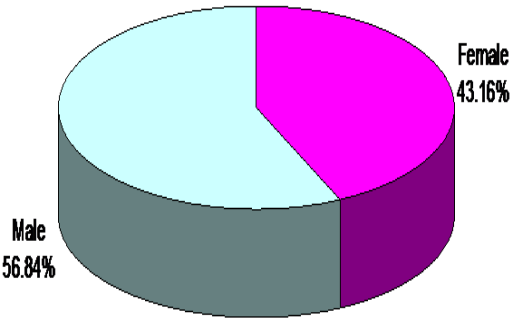


The present study consisted of 95 patients, out of these majority were children <16 years (65.26%), 20 (21.05%) were aged 16-45 years and 13 (13.68%) were aged >45 years. We know that AML is more common in adults, contrary to our findings here. Explaination to this finding lies in the fact that in our institution, there is more admission of pediatric patients.

Also these findings came out to be statistically insignificant.

Table 2: Distribution of Study Population according to Gender (N=95)

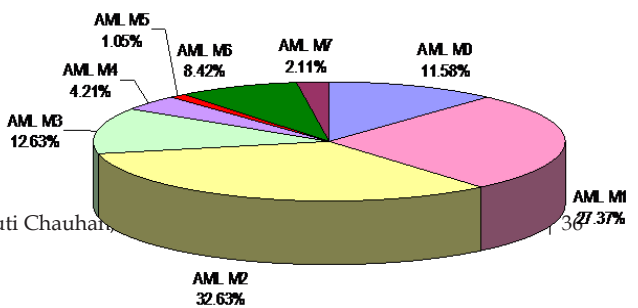
Gender	Number	Percentage
Female	41	43.16
Male	54	56.84



Prevalence was found to be higher in males (56.84%)as compared to females (43.16%). Male:Female ratio was 1.3:1.(TABLE 2).

Table 3a: Distribution of Study Population according to Morphological Diagnosis (N=95)

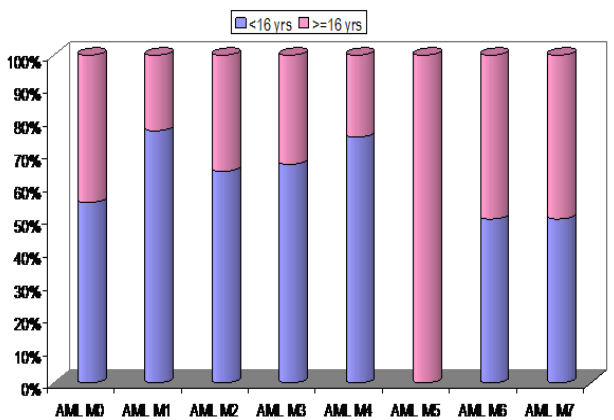
Morphological Diagnosis	Number	Percentage
AML M0	11	11.58
AML M1	26	27.37
AML M2	31	32.63
AML M3	12	12.63
AML M4	4	4.21
AML M5	1	1.05
AML M6	8	8.42
AML M7	2	2.11



Most common morphological diagnosis was AML-M2 (32.63%) followed by AML-M1 (27.37%), AML-M3 (12.63%), AML-M0 (11.58%), AML-M6 (8.42%), AML-M4 (4.21%), AML-M7 (2.11%) and least common was AML-M5 (1.05%).

Table 3(b): Comparison of morphological diagnosis in Children and adults

Morphological Diagnosis	Total (N=95)		Age <16 yrs (n=62) Children		Age >16 (n=33) Adults	Statistical Significance	
	No.	%	No.	%	χ^2	P	
AML M0	11	6	55.54	5	45.45	5.206 (df=7)	0.635
AML M1	26	20	76.92	6	23.08		
AML M2	31	20	64.52	11	35.48		
AML M3	12	8	66.67	4	33.33		
AML M4	4	3	75.00	1	25.00		
AML M5	1	0	0.00	1	100.00		
AML M6	8	4	50.00	4	50.00		
AML M7	2	1	50.00	1	50.00		



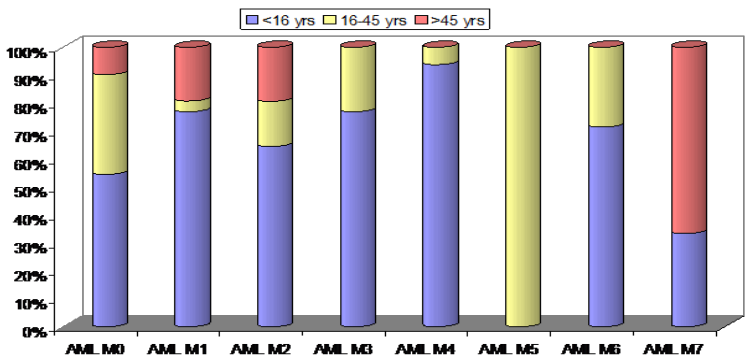
Though proportion of subjects aged <16 years was high in AML-M1 (76.92%) and AML-M4 (75.00%) as compared to subjects with other morphological

diagnosis but this difference was not found to be statistically significant (p=0.635).As per the table, maximum number of cases were from AML M1 and M2, common subtype in the pediatric population while AML M5 is the least common. Also, AML M2 had maximum number of cases in the adult population while AML M4,5 and 7 are the less common subtypes.

Table 3(c): Comparison of morphological diagnosis in different age groups

Morphological Diagnosis	Total (N=95)		Age <16 yrs (n=62)		Age 16-45 yrs (n=20)		>45 yrs (n=13)	
	No.	%	No.	%	No.	%		
AML M0	11	6	55.54	4	36.36	1	9.99	
AML M1	26	20	76.92	1	3.85			
5	19.23							
AML M2	31	20	64.52	5	16.13	6	19.35	
AML M3	12	8	66.67	4	20.00	0	0.00	
AML M4	4	3	75.00	1	5.00	0	0.00	
AML M5	1	0	0.00	1	5.00	0	0.00	
AML M6	8	4	50.00	4	20.00	0	0.00	
AML M7	2	1	50.00	0	0.00	1	100.00	

21.334=^{2} (df=14); p=0.093 (Non-Significant)

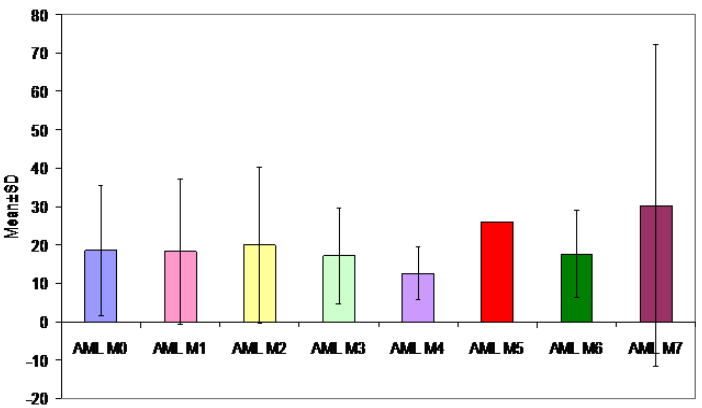


Majority of patients of all the morphological diagnosis were <16 years of age except the only patient diagnosed as AML-M5 (n=1) who was aged between 16-45 years. Difference in age of patients with different morphological diagnosis was not found to be statistically significant (p=0.093).In the age group >45 years, the proportion was highest in AML M7 (100%) but maximum number of cases were seen in AML M2.

Table 3(d): Comparison of Age in different morphological diagnoses

Morphological Diagnosis	No. of subjects	Min.	Max.	Mean	S.D.
AML M0	11	1.50	47.00	18.59	16.89
AML M1	26	1.50	65.00	18.29	18.96
AML M2	31	1.50	80.00	19.97	20.24
AML M3	12	3.00	42.00	17.17	12.47
AML M4	4	7.00	22.00	12.50	6.86
AML M5	1	26.00	26.00	26.00	.
AML M6	8	2.00	35.00	17.63	11.33
AML M7	2	0.58	60.00	30.29	42.01
Total	95	0.58	80.00	18.76	17.63

F=0.246; p=0.972



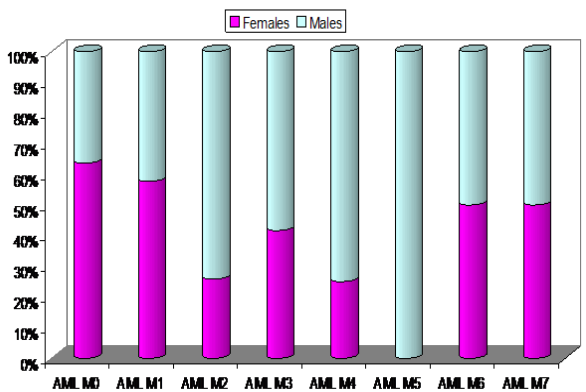
Age of patients ranged from 7 months to 80 years with a mean age of 18.76 and standard deviation of 17.63 years. Table 3d shows that the minimum age was seen in AML M7 while the maximum was seen in M2 subtype. The highest mean is seen in the AML M5 subtype.

Age of patients of AML-M7 (30.29±42.01 years) and AML-M5 (26.00 years; n=1) was found to be higher than that having other morphological diagnosis. Minimum mean age was found to be in AML-M4 patients.

Difference in mean age of patients with different diagnosis was not found to be statistically significant (p=0.972).

Table 4: Comparison of morphological diagnosis in Females and Males

Morphological Diagnosis	Total (N=95)		Female (n=41)		Male (n=54)	
Statistical Significance						
	No.	%	No.	%	^{2}	p
AML M0 11	7	63.64	4	36.36	9.422 (df=7)	0.224 (NS)
AML M1 26	15	57.69	11	42.31		
AML M2 31	8	25.81	23	74.19		
AML M3 12	5	41.67	7	58.33		
AML M4 4	1	25.00	3	75.00		
AML M5 1	0	0.00	1	100.00		
AML M6 8	4	50.00	4	50.00		
AML M7 2	1	50.00	1	50.00		

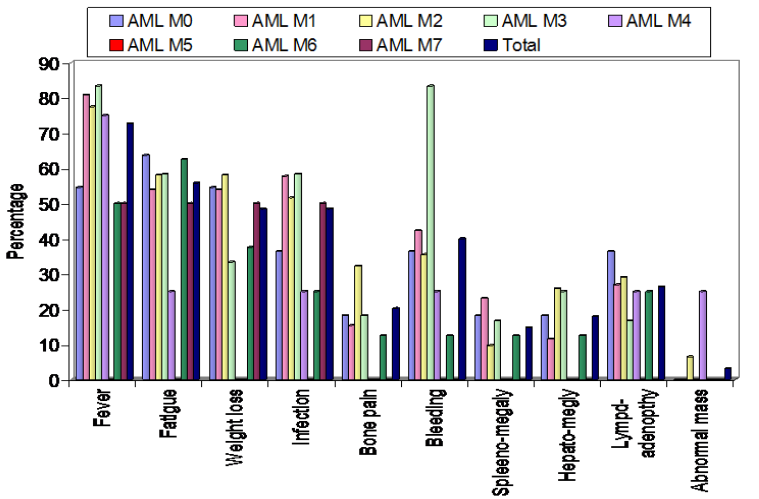


Majority of patients of AML-M0 and AML-M1 were females while majority of patients diagnosed as AML-M2, AML-M3 and AML-M4 were males. There was equal gender based distribution in AML M6 and M7. Only patient diagnosed as AML-M5 was male. Despite proportional difference in gender of subjects with different morphological diagnosis the difference was not found to be statistically significant (p=0.224).

Table 5: Comparison of Presenting Symptoms and signs in different morphological diagnoses

	M0 (n=11)		M1 (n=26)		M2 (n=31)		M3 (n=12)		M 4
(n=4)	M5 (n=1)	M6 (n=8)	M7 (n=2)	Total (N=95)		^{2}	p		
Fever	6	21	24	10	3	0	4	1	69
8.967	0.255								
	54.55	80.77	77.42	83.33	75.00	0.00	50.00	50.00	
72.63									
Fatigue	7	14	18	7	1	0	5	1	53
3.383	0.847								
	63.64	53.85	58.06	58.33	25.00	0.00	62.50	50.00	
55.79									
Weight loss		6	14	18	4	0	0	3	1
46	7.798	0.351							
	54.55	53.85	58.06	33.33	0.00	0.00	37.50	50.00	
48.42									
Infection	4	15	16	7	1	0	2	1	46
5.710	0.574								
	36.36	57.69	51.61	58.33	25.00	0.00	25.00	50.00	
48.42									
Bone pain	2	4	10	2	0	0	1	0	19
5.289	0.625								
	18.18	15.38	32.26	18.18	0.00	0.00	12.50	0.00	
20.21									
Bleeding	4	11	11	10	1	0	1	0	38
14.666	0.041								
	36.36	42.31	35.48	83.33	25.00	0.00	12.50	0.00	
40.00									

Splenomegaly	2	6	3	2	0	0	1	0
14	3.452	0.840						
	18.18	23.08	9.68	16.67	0.00	0.00	12.50	0.00
14.74								
Hepatomegaly	2	3	8	3	0	0	1	0
17	4.133	0.764						
	18.18	11.54	25.81	25.00	0.00	0.00	12.50	0.00
17.89								
Lymphadenopathy	4	7	9	2	1	0	2	0
25	2.354	0.938						
	36.36	26.92	29.03	16.67	25.00	0.00	25.00	0.00
26.32								
Abnormal mass								
(orbital swelling)	0	0	2	0	1	0	0	0
3	9.301	0.232						
	0.00	0.00	6.45					
0.00	25.0	0.00	0.00	0.00	3.16			



Overall most common symptom was fever (72.63%) followed by Fatigue (55.79%), weight loss (48.42%) and infection (48.42%), the less common ones were bleeding, lymphadenopathy, bone pain, hepatomegaly and splenomegaly while the least common symptom was abnormal mass

(3.16%).

Fever was present in 69 (72.63%) subjects. Proportion of patients presenting with fever was higher in AML M3 (83.33%), AML M1 (80.77%), AML M2 (77.42%) and AML M4 (75.00%) as compared to patients with other morphological diagnosis (0 to 54.55%). Difference in fever as presenting symptom in different morphological diagnosis was not found to be statistically significant (p=0.255).

Fatigue was present in 53 (55.79%) subjects. Proportion of patients presenting with fatigue was higher in AML M0 (63.64%) AML M6 (62.50%) and AML-M2 (58.06%) as compared to patients with other morphological diagnosis (0 to 55.79%). Difference in fatigue as presenting symptom in different morphological diagnosis was not found to be statistically significant (p=0.847).

Weight loss was present in 46 (48.42%) subjects. Proportion of patients presenting with weight loss was higher in AML M2 (58.06%), AML M0 (54.55%), AML M1 (53.85%) as compared to patients with other

Diagnosis. However, this was not found to be statistically significant (p=0.574).

Bone pain was found in 19 (20.21%). Proportional differences in bone pain as presenting symptoms in different morphological diagnosis was not found to be statistically significant (p=0.625).

Bleeding was found in 38 (40.00%) patients. Proportion of patients diagnosed as AML M3 (83.33%) was found to be significantly higher than other morphological diagnosis (p=0.041%). This was followed by AML M1 and M0 and least in AML M5 and M7 types.

Splenomegaly was found in 14 (14.74%) patients. Proportional differences in splenomegaly as presenting symptoms in different morphological diagnosis was not found to be statistically significant (p=0.840). It was most common in AML M1 subtype followed by AML M0 and least common in AML M4, M5 and M7.

Hepatomegaly was found in 17 (17.89%) patients. Proportional differences in hepatomegaly as presenting symptoms in different morphological diagnosis was not found to be statistically

significant (p=0.764)

It was most common in AML M2 and M3 subtype and least common in AML M4 and M5.

Lymphadenopathy was found in 25 (26.32%) patients. Proportional differences in Lymphadenopathy as presenting symptoms in different morphological diagnosis was not found to be statistically significant (p=0.938).

It was most commonin AML M0 subtype followed by AML M2 and M1 and least common in AML M5 and M7.

Abnormal mass (i.e. orbital swelling) was found in only 3 (3.16%) patients. Abnormal mass was observed in 2 (6.45%) patients of AML-M2 and 1 (25.0%) patients of AML-M4. Proportional differences in abnormal mass as presenting symptoms in above two morphological diagnosis was not found to be statistically significant (p=0.232).

B. Morphological and cytochemical studies:

Table 6a: Comparison of Hematological Variables in different morphological diagnoses

Variable	Morphological Diagnosis	No. of subjects			Min.	Max.	Mean
S.D.	F	p					
PBS-Hb	AML M0	11	3.90	11.00	6.80	2.47	0.853	0.547
	AML M1	26	3.00	9.00	5.70	1.56		
	AML M2	31	2.60	12.60	6.14	2.21		
	AML M3	12	4.10	10.30	6.18	1.69		
	AML M4	4	4.40	5.10	4.73	0.33		
	AML M5	1	4.00	4.00	4.00	.		
	AML M6	8	5.30	9.10	6.23	1.30		
	AML M7	2	5.60	7.10	6.35	1.06		
	Total	95	2.60	12.60	6.03	1.89		
TLC	AML M0	11	3300	410000	68318	118885	0.963	0.464
	AML M1	26	1200	160000	31242	40005		
	AML M2	31	1100	230000	45655	55668		
	AML M3	12	3500	112000	22975	31304		

	AML M4	**4**	**28000**	**81600**	**46300**	**24355**		
	AML M5	**1**	**11600**	**11600**	**11600**	**.**		
	AML M6	**8**	**1800**	**22400**	**14525**	**8460**		
	AML M7	**2**	**9000**	**21000**	**15000**	**8485**		
	Total	**95**	**1100**	**410000**	**37872**	**57400**		
PC	**AML M0**	**11**	**10000**	**78000**	**26182**	**18324**	**0.699**	**0.673**
	AML M1	**26**	**8000**	**170000**	**42150**	**39697**		
	AML M2	**31**	**8000**	**245000**	**44116**	**54238**		
	AML M3	**12**	**10000**	**48000**	**23450**	**11276**		
	AML M4	**4**	**10000**	**55000**	**35250**	**23243**		
	AML M5	**1**	**35000**	**35000**	**35000**	**.**		
	AML M6	**8**	**20000**	**160000**	**53625**	**50746**		
	AML M7	**2**	**18200**	**110000**	**64100**	**64912**		
	Total	**95**	**8000**	**245000**	**39643**	**41903**		

The haemoglobin levels ranged from 2.6 to 12.6 gm% with a mean value of 6.03 gm% ±1.89(SD). The lowest as well as the highest value was found in AML M2 subtype. AML M5 had the lowest mean value while AML M0 had the highest mean value.

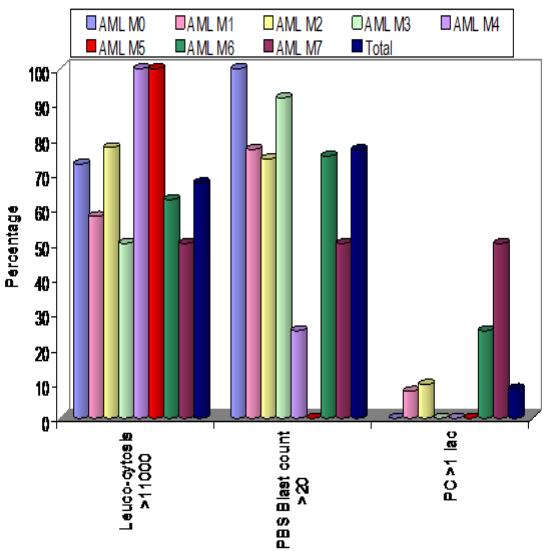
The hemoglobin levels were found to be <10 gm/dl in 91 (95.79%) patients while in 4 patients (4.21%) hemoglobin levels were found to be >10 gm/dl. Out of these 4 patients, 1 was diagnosed as AML-M0, 2 as AML M2 and 1 as AML-M3.

The total leukocyte count ranged from 1100 cells/cu.mm to 4,10,000 cells/cu.mm.The mean value was 37,872 cell/cu.mm ±57400 cells/cu.mm(SD). The lowest value was seen in AML M2 while the highest value was found in AML M0 subtype.AML M5 had the lowest mean value while the AML M0 had the highest mean value.

The platelet count ranged from 1100 /cu.mm to 4,10,000 / cu.mm.The mean value was 37,872 /cu.mm ±57400 /cu.mm(SD). The lowest value was seen in AML M2 while the highest value was found in AML M0 subtype.AML M5 had the lowest mean value while the AML M0 had the highest mean value.

Table 6b: Comparison of Hematological Variables in different morphological diagnoses

	M0 (n=11)		M1 (n=26)		M2 (n=31)		M3 (n=12)		M 4
(n=4)	M5 (n=1)	M6 (n=8)	M7 (n=2)	Total (N=95)		^{2}	p		
Leucocytosis >11000cells/cumm			8	15	24	6	4	1	
5	1	64	7.105	0.418					
	72.73	57.69	77.42	50.00	100.00	100.00	62.50	50.00	67.37
PBS Blast count >20%			11	20	23	11	1	0	6
1	73	15.104	0.035						
	100.00	76.92	74.19	91.67	25.00	0.00	75.00	50.00	76.84
PC >1 lac/cumm	0	2	3	0	0	0	2	1	
8	9.991	0.189							
	0.00	7.69	9.68	0.00	0.00	0.00	25.00	50.00	8.42



Leucocytosis (>11,000 cells/ cu.mm) was found in 64 (67.37%) patients. Proportion of patients with leucocytosis was found in higher proportion of patients of AML-M4 (100.0%), AML-M5 (n=1; 100.0%), AML-M2 (77.42%) and

AML-M0 (72.73%) as compared to patients with other morphological diagnosis but this difference was not found to be statistically significant (p=0.418).

Leucocytopenia (<11,000/ cu.mm) was found in 31 (32.63%) patients of the study population. Proportion of patients with leucocytopenia was found in higher proportion of patients of AML-M3 (50.0%), AML-M7 (50.0%) and AML-M1 (42.31%) as compared to patients with other morphological diagnosis.

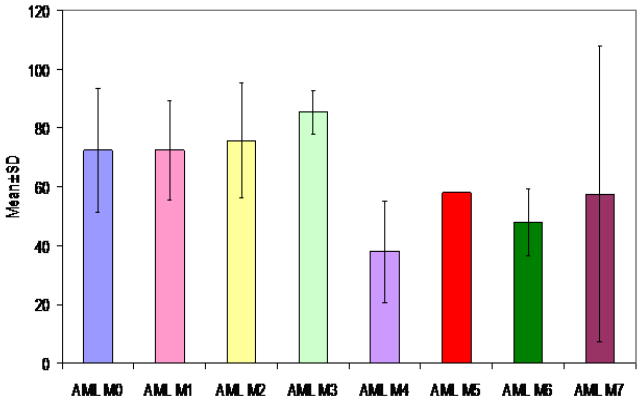
PBS-blast count (>20%) was found in 73 (76.84%) patients. Proportion of patients with PBS-blast count >20% was highest in AML M0 and AML M3 and significantly lower in AML-M4 (25.0%) as compared to patients of other morphological diagnosis. The single patient diagnosed as AML-M5 had PBS-blast count <20% (ie was in aleukemic phase).

Platelet count >1 lac were found in only 8 (8.42%) patients. None of the patients in AML M0, M3, M4, and M5 had platelet count more than 1 lac. Thus, all the cases in these subtypes showed thrombocytopenia. Though proportion of patients with platelet count >1 lac was higher in AML-M7 (50.0%) and AML-M6 (25.00%) as compared to patients with other morphological diagnosis but this difference was not found to be statistically significant.

Table 7: Comparison of Bone Marrow Blast Cells in different morphological diagnoses

Morphological Diagnosis	No. of subjects	Min.	Max.	Mean	S.D.
AML M0	11	23.00	93.00	72.45	21.14
AML M1	26	37.00	95.00	72.42	16.76
AML M2	31	28.00	98.00	75.77	19.43
AML M3	12	71.00	97.00	85.33	7.27
AML M4	4	21.00	62.00	38.00	17.26
AML M5	1	58.00	58.00	58.00	.
AML M6	8	23.00	61.00	48.00	11.36
AML M7	2	22.00	93.00	57.50	50.20
Total	95	21.00	98.00	71.18	20.65

F=5.547; p<0.001



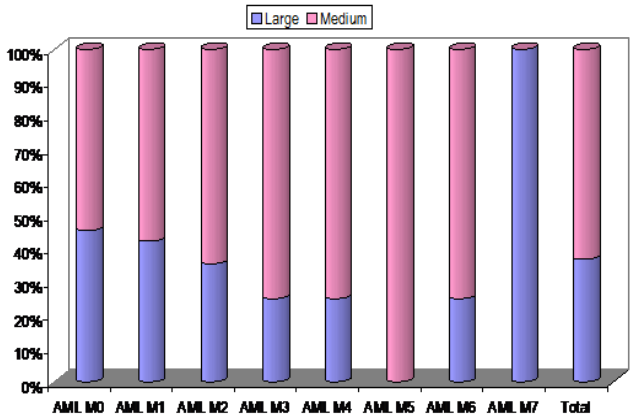
Number of bone marrow blast cells were found to be significantly lower in patients diagnosed as AML M4 (38.00+17.26), AML M5 (58.00) and AML M6 (57.50+50.20) as compared to patients with other diagnosis. None of the patient had blast count <20%.

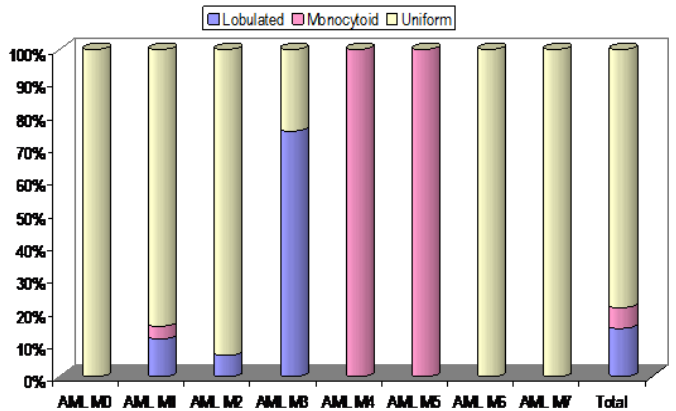
Table 8: Comparison of Morphology of Blasts in different morphological diagnoses

	M0 (n=11)	M1 (n=26)	M2 (n=31)	M3 (n=12)	M4 (n=4)	M5 (n=1)	M6 (n=8)	M7 (n=2)	Total (N=95)	χ^2	P
Size											
Large	5	11	11	3	1	0	2	2	35	6.167	0.520
	45.45	42.31	35.48	25.00	25.00	0.00	25.00	100.00	36.84		
Medium	6	15	20	9	3	1	6	0	60		
	54.55	57.69	64.52	75.00	75.00	100.00	75.00	0.00	63.16		
Shape											
Lobulated		0	3	2	9	0	0	0	14	119.297	<0.001
	0.00	11.54	6.45	75.00	0.00	0.00	0.00	0.00			

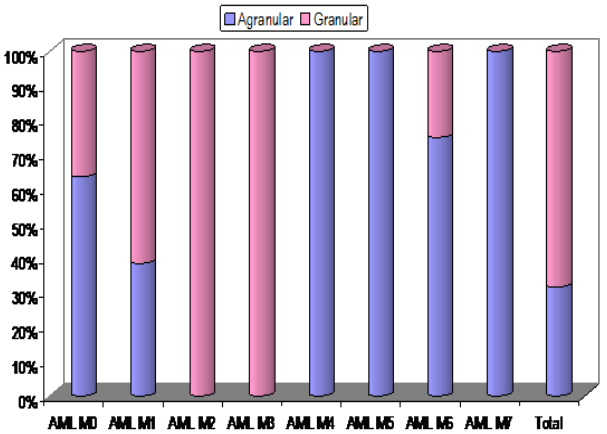
14.74									
Monocytoid		0	1	0	0	4	1	0	0
6									
	0.00	3.85	0.00	0.00	100.00	100.00	0.00	0.00	6.32
Uniform	11	22	29	3	0	0	8	2	75
	100.00	84.62	93.55	25.00	0.00	0.00	100.00	100.00	78.95
Cytoplasm									
Agranular		7	10	0	0	4	1	6	2
30	47.796	<0.001							
	63.64	38.46	0.00	0.00	100.00	100.00	75.00	100.00	31.58
Granular	4	16	31	12	0	0	2	0	65
	36.36	61.54	100.00	100.00	0.00	0.00	25.00	0.00	68.42
Dysplasia									
Dysplasia	0	2	6	1	0	0	0	0	9	6.365
0.498									
	0.00	7.69	19.35	8.33	0.00	0.00	0.00	0.00	9.47
No dysplasia	11	24	25	11	4	1	8	2	86
	100.00	92.31	80.65	91.67	100.00	100.00	100.00	100.00	91.53

Out of 95 patients included in the study in majority cases (n=60; 63.16%) , blast size was found to be medium. All the patients diagnosed as AML-M7 had larger blast size as compared to other cases. Despite proportional difference in size of blasts in patients with different morphological diagnoses, this difference was not found to be statistically significant (p=0.520).

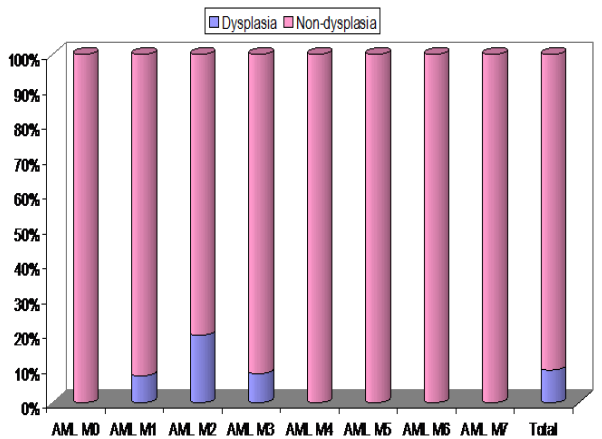




Shape of blasts in majority of subjects in our study was uniform (n=75; 78.95%). All the subjects diagnosed as AML-M4 and AML-M5 had predominantly monocytoid shape. Lobulated shape was found in most of the patients (9 ;75%) diagnosed as AML-M3. Difference in shapes of blasts in patients with different morphological diagnosis was found to be statistically significant ($p<0.001$).



Cytoplasm was found to be granular in majority of patients of our study (n=65; 68.42%). All the patients diagnosed as AML-M2, AML-M3 had granular cytoplasm while the patients of AML-M4, AML-5 and AML-M7 had agranular cytoplasm. Difference in granularity of cytoplasm in patients with different morphological diagnosis was found to be statistically significant ($p<0.001$).

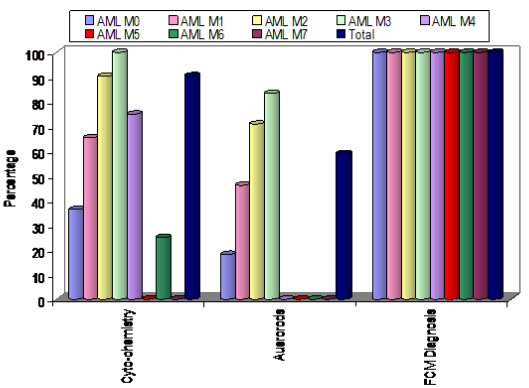


Dysplasia was found in 9 (9.47%) patients. None of the patients diagnosed as AML-M0, AML-M4, AML-M5, AML-M6 and AML-M7 had dysplasia. Dysplasia was found in higher proportion of patients diagnosed as AML-M2 (19.35%) as compared to

AML-M3 (8.33% and AML-M1 (7.69%).These cases of AML may have been the ones that had progressed from myelodysplastic syndrome. Difference in dysplasia in patients with different morphological diagnosis was not found to be statistically significant (p=0.498).

Table 9: Comparison of Positive Cytochemistry and Auer Rods in different morphological diagnoses

	M0 (n=11)	M1 (n=26)	M2 (n=31)	M3 (n=12)	M4 (n=4)	M5 (n=1)	M6 (n=8)	M7 (n=2)	Total	p
Cyto-chemistry	4	17	28	12	3	0	2	0	66/95	31.864	<0.001
6.36	65.38	90.32	100.00	75.00	0.00	25.00	0.00	69.47			
	4	17	28	12	3				64/84		
76.19%											
Auer rods	2	12	22	10	0	0	0	0	46	30.329	<0.001
	18.18	46.15	70.97	83.33	0.00	0.00	0.00	0.00	48.42		
46/84											
54.74%											
FCM Diagnosis	11	26	31	12	4	1	8	2	95		
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00		



The cytochemical staining that was done using Myeloperoxidase (MPO) method was found to be positive in 64/84 cases (76.19%), excluding the AML M5, M6 and M7 cases.Maximum positivity was seen in AML M3(100%) category, followed by AML M2(90.32%). Statistically, the positivity of MPO in identifying the myeloid lineage was found to be significant. ($p<0.001$)

Auer rods were found to be positive in 46/84 cases excluding the AML M5, M6 and M7 subtypes (54.74%). Maximum positivity was seen in AML M3(83.3%) category, followed by AML M2(70.97%) , M1 (46.12%) and M0 (18.18%) while not seen in any case of AML M4,M5,M6 and M7(0%).

Table10: Immune markers in different morphological diagnoses (mean±SD)

Variable	Morphological Diagnosis	No. of subjects		Min.	Max.	Mean	S.D.	F	P
CD13	AML M0	11	0.00	94.00	69.55	32.74	0.514	0.796	
	AML M1	24	0.00	96.00	62.15	30.11			
	AML M2	27	7.00	99.00	69.79	27.74			
	AML M3	10	0.00	98.00	75.49	29.71			
	AML M4	4	65.00	93.00	81.70	11.86			
	AML M6	6	43.00	98.00	62.05	23.84			
	AML M7	1	58.40	58.40	58.40	.			
	Total	83	0.00	99.00	68.11	28.23			
CD33	AML M0	11	8.00	97.00	73.34	25.54	1.860	0.099	
	AML M1	24	7.00	99.00	75.55	26.43			
	AML M2	27	0.00	99.00	75.33	25.87			
	AML M3	10	83.00	99.00	94.79	4.63			
	AML M4	4	45.00	80.00	64.25	15.00			
	AML M6	6	85.00	98.30	95.72	5.27			
	AML M7	1	80.90	80.90	80.90	.			
	Total	83	0.00	99.00	78.48	23.95			
HLADR	AML M0	5	0.00	95.00	73.96	41.42	4.603	0.001 (Sig)	
	AML M1	11	0.00	92.00	66.55	25.66			
	AML M2	17	0.00	99.60	59.78	31.88			
	AML M3	10	0.00	54.00	9.90	20.98			
	AML M4	4	0.00	69.00	44.50	30.47			
	AML M6	5	0.00	81.20	62.20	34.84			
	AML M7	1	76.00	76.00	76.00	.			
	Total	53	0.00	99.60	52.49	35.66			

MPO	AML M0	8	0.00	83.00	36.88	32.45	4.137	0.002
	AML M1	18	0.00	89.00	48.22	28.33		
	AML M2	17	0.00	94.00	61.51	25.26		
	AML M3	8	31.00	99.30	74.41	20.57		
	AML M4	3	0.00	66.00	32.67	33.01		
	AML M6	6	0.00	85.10	14.18	34.74		
	AML M7	1	0.00	0.00	0.00	.		
	Total	61	0.00	99.30	48.97	32.12		
CD3	AML M0	5	0.00	1.00	0.24	0.43	1.404	0.257
	AML M1	12	0.00	7.00	1.44	2.11		
	AML M2	11	0.00	2.00	0.30	0.64		
	AML M3	5	0.10	2.00	0.54	0.82		
	AML M4	2	0.00	0.00	0.00	0.00		
	AML M6	0	.	.	.	.		
	AML M7	0	.	.	.	.		
	Total	35	0.00	7.00	0.70	1.40		
CD2	AML M0	10	0.00	34.00	3.42	10.74	0.531	0.752
	AML M1	16	0.00	97.00	8.75	25.46		
	AML M2	21	0.00	32.00	1.62	6.97		
	AML M3	4	0.00	46.00	11.50	23.00		
	AML M4	2	0.00	4.00	2.00	2.83		
	AML M6	2	0.00	0.00	0.00	0.00		
	AML M7	0	.	.	.	.		
	Total	55	0.00	97.00	4.69	16.13		
CD7	AML M0	11	0.00	78.00	15.27	27.37	1.035	0.410
	AML M1	22	0.00	85.00	21.35	31.76		
	AML M2	25	0.00	65.10	4.55	14.16		
	AML M3	9	0.00	94.70	10.71	31.50		
	AML M4	3	0.00	0.10	0.03	0.06		
	AML M6	6	0.00	65.00	11.13	26.39		
	AML M7	1	1.60	1.60	1.60	.		
	Total	77	0.00	94.70	11.90	25.36		
CD10	AML M0	11	0.00	0.10	0.01	0.03	0.429	0.827
	AML M1	21	0.00	3.20	0.35	0.94		
	AML M2	20	0.00	97.00	5.13	21.65		
	AML M3	8	0.00	2.00	0.36	0.73		

	AML M4	2	0.00	0.00	0.00	0.00		
	AML M6	2	0.00	0.00	0.00	0.00		
	AML M7	0	.	.	.	.		
	Total	64	0.00	97.00	1.76	12.12		
CD19	AML M0	10	0.00	72.00	28.41	36.04	0.989	0.431
	AML M1	22	0.00	77.00	19.09	25.35		
	AML M2	23	0.00	88.70	24.57	33.56		
	AML M3	8	0.00	63.00	8.69	21.99		
	AML M4	2	0.00	50.00	25.00	35.36		
	AML M6	5	0.00	0.50	0.30	0.27		
	AML M7	0	.	.	.	.		
	Total	70	0.00	88.70	19.86	29.20		
CD117	AML M0	10	0.00	87.00	45.84	34.01	2.766	0.018 (Sig)
	AML M1	23	0.00	99.00	61.37	29.40		
	AML M2	27	0.00	94.30	59.18	25.67		
	AML M3	9	0.00	82.00	30.17	34.02		
	AML M4	4	0.00	59.80	23.45	29.05		
	AML M6	6	54.00	97.00	72.47	17.32		
	AML M7	1	45.40	45.40	45.40	.		
	Total	80	0.00	99.00	53.92	30.49		
CD34	AML M0	11	48.00	94.00	74.06	14.55	5.744	<0.001 (Sig.)
	AML M1	22	0.00	98.00	66.32	34.64		
	AML M2	24	0.00	99.00	69.43	34.25		
	AML M3	10	0.00	53.00	10.45	22.03		
	AML M4	4	0.00	86.00	50.98	36.38		
	AML M6	6	0.00	96.00	48.00	39.89		
	AML M7	1	0.00	0.00	0.00	.		
	Total	78	0.00	99.00	58.16	36.90		
CD16	AML M0	0	.	.	.	.	2.039	0.254
	AML M1	1	0.30	0.30	0.30	.		
	AML M2	0	.	.	.	.		
	AML M3	1	0.10	0.10	0.10	.		
	AML M4	2	1.50	6.60	4.05	3.61		
	AML M6	4	0.70	4.10	2.95	1.53		
	AML M7	1	7.80	7.80	7.80	.		
	Total	9	0.10	7.80	3.12	2.76		

CD14	AML M0	0	.	.	.	.	0.973	0.494
	AML M1	1	14.20	14.20	14.20	.		
	AML M2	2	0.10	3.00	1.55	2.05		
	AML M3	1	0.20	0.20	0.20	.		
	AML M4	4	12.70	87.00	35.45	34.62		
	AML M6	4	6.10	11.20	7.58	2.44		
	AML M7	1	8.50	8.50	8.50	.		
	Total	13	0.10	23.00	9.55	7.08		
CD64	AML M0	1	14.20	14.20	14.20	.	0.668	0.663
	AML M1	1	3.90	3.90	3.90	.		
	AML M2	2	4.10	19.00	11.55	10.54		
	AML M3	0	.	.	.	.		
	AML M4	3	21.80	93.00	46.37	40.40		
	AML M6	4	16.70	61.00	28.88	21.45		
	AML M7	1	7.80	7.80	7.80	.		
	Total	12	3.90	93.00	25.30	25.94		
CD36	AML M0	1	6.00	6.00	6.00	.	19.140	0.001
	AML M1	0	.	.	.	.		
	AML M2	0	.	.	.	.		
	AML M3	1	0.30	0.30	0.30	.		
	AML M4	4	19.00	27.00	24.43	3.66		
	AML M6	4	19.00	24.90	22.53	2.52		
	AML M7	1	10.50	10.50	10.50	.		
	Total	11	0.30	27.00	18.60	9.03		
CD11b	AML M0	0	.	.	.	.	17.223	0.025
	AML M1	0	.	.	.	.		
	AML M2	0	.	.	.	.		
	AML M3	0	.	.	.	.		
	AML M4	3	21.00	41.30	31.43	10.16		
	AML M6	2	0.00	0.00	0.00	0.00		
	AML M7	0	.	.	.	.		
	Total	5	0.00	41.30	18.86	18.66		
CD15	AML M0	0	.	.	.	.	–	–
	AML M1	0	.	.	.	.		
	AML M2	0	.	.	.	.		
	AML M3	1	34.00	34.00	34.00	.		

AML M4	1	71.00	71.00	71.00	.
AML M6	0	.	.	.	.
AML M7	0	.	.	.	.
Total	2	34.00	71.00	52.50	26.16

Mean expression of HLADR of patients with different morphological diagnosis (n=53) was found to be statistically significant (p=0.001). HLADR expression was found to be lower in patients diagnosed as AML-M3 (9.90±20.98%) and as AML-M4 (44.50±30.47%) as compared to patients with other morphological diagnoses.

Mean expression of MPO of patients with different morphological diagnosis (n=61) was found to be statistically significant (p=0.002). MPO expression was found to be 0.00% in patients diagnosed as AML-M7 and was lower in patients diagnosed as AML-M6 (14.18± 34.74%), AML-M4 (32.67± 33.01%) and AML-M0 (36.88± 32.45%) as compared to patients with other morphological diagnoses.

Mean expression of CD117 of patients with different morphological diagnosis (n=80) was found to be statistically significant (p=0.018). CD117 expression was found to be lower in patients diagnosed as AML-M4 (23.45±29.05%) and as AML-M3 (30.17±34.02%), AML-M7 (45.40%), AML-M0 (45.84±34.01%) as compared to patients with other morphological diagnosis.

Mean expression of CD34 of patients with different morphological diagnosis (n=78) was found to be statistically significant (p<0.001). CD34 expression in patients diagnosed as AML-M7 was found to be 0.00% , was found to be lower in patients diagnosed as AML-M3 (10.45±22.03%) as compared to patients with other morphological diagnosis.

Mean expression of CD14 of patients with different morphological diagnosis (n=13) was not found to be statistically significant (p=0.494). CD14 expression was found to be lower in patients diagnosed as AML-M3 (0.20%), AML-M2 (1.55±2.05%) as compared to AML-M4 (35.45±34.62%), AML-M1 (14.20%), AML-M7 (8.505), AML-M6 (7.58±2.54%).

Mean expression of CD36 of patients with different morphological diagnosis (n=11) was found to be statistically

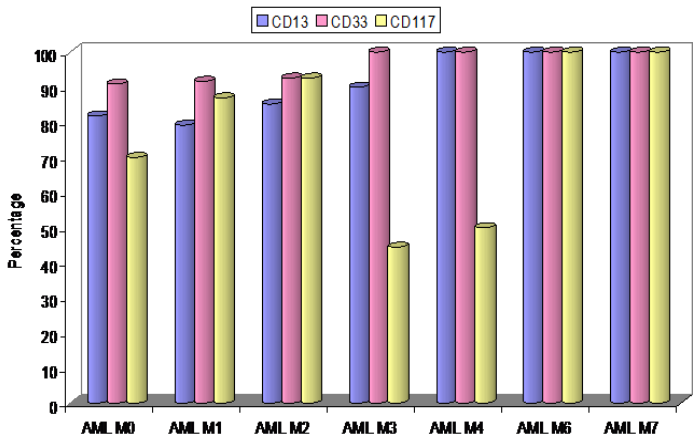
significant (p=0.001). CD36 expression was found to be lower in patients diagnosed as AML-M3 (0.30%), AML-M0 (6.00%) as compared to patients with other morphological diagnosis.

Mean expression of CD11b was done in 5 patients, expression in 2 patients diagnosed as AML-M6 was 0.00% while in patients diagnosed as AML-M4 was found to be 31.43+10.16%. Difference in CD11b expression in patients diagnosed as AML-M6 and AML-M4 was found to be statistically significant (p=0.025).

Table 11a: Comparison of Immune Markers positivity in different morphological diagnosis

	M0	M1	M2	M3	M4	M6	M7	Tota	²	P
CD13										
<20%	2	5	4	1	0	0	0	12	2.935	0.817
	18.18	20.83	14.81	10.00	0.00	0.00	0.00	14.46		
>20%	9	19	23	9	4	6	1	71		
	81.82	79.17	85.19	90.00	100.00	100.00	100.00	85.54		
Total	11	24	27	10	4	6	1	83		
CD33										
<20%	1	2	2	0	0	0	0	5	1.846	0.933
	9.09	8.33	7.41	0.00	0.00	0.00	0.00	6.02		
>20%	10	22	25	10	4	6	1	78		
	90.91	91.67	92.59	100.00	100.00	100.00	100.00	93.98		
Total	11	24	27	10	4	6	1	83		
CD117										
<20%	3	3	2	5	2	0	0	15		

15.785	0.015							
	30.00	13.04	7.41	55.56	50.00	0.00	0.00	
18.75								
>20%	7	20	25	4	2	6	1	65
	70.00	86.96	92.59	44.44	50.00	100.00	100.00	
81.25								
Total	10	23	27	9	4	6	1	80



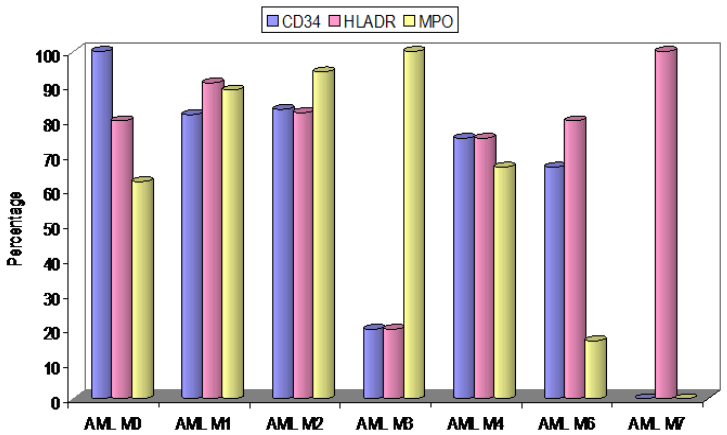
The above table shows that expression of marker CD13 was seen in 85.54% of cases (71/83). Maximum positivity was seen in M4,M6 and M7 (100% cases) of AML followed by AML M3, M2, M0 and M1.

The above table shows that expression of marker CD33 was seen in 93.98% of cases (78/83). Maximum positivity was seen in M3,M4,M6 and M7 (100% cases) of AML followed by AML M2, M1 and M0.

The above table shows that expression of marker CD117 was seen in 81.25% of cases (65/80). Maximum positivity was seen in M6 and M7 (100% cases) of AML followed by AML M2, M1, M0, M4 and M3. These findings came out to be significant.

Table 11b: Comparison of Immune Markers positivity in different morphological diagnoses

	M0	M1	M2	M3	M4	M6	M7	Total
^{2}	P							
HLADR								
<20%	1	1	3	8	1	1	0	15
16.879	0.010							
	20.00	9.09	17.65	80.00	25.00	20.00	0.00	28.30
>20%	4	10	14	2	3	4	1	38
	80.00	90.91	82.35	20.00	75.00	80.00	100.00	71.70
Total	5	11	17	10	4	5	1	53
MPO								
<10%	3	2	1	0	1	5	1	13
24.661	0.000							
	37.50	11.11	5.88	0.00	33.33	83.33	100.00	21.31
>10%	5	16	16	8	2	1	0	48
	62.50	88.89	94.12	100.00	66.67	16.67	0.00	78.69
Total	8	18	17	8	3	6	1	61
CD34								
<20%	0	4	4	8	1	2	1	20
24.034	0.001							
	0.00	18.18	16.67	80.00	25.00	33.33	100.00	25.64
>20%	11	18	20	2	3	4	0	58
	100.00	81.82	83.33	20.00	75.00	66.67	0.00	74.36
Total	11	22	24	10	4	6	1	78



The above table shows that expression of marker CD34 was seen in 74.36% of cases (58/78). Maximum positivity was seen in M0 (100% cases) of AML followed by AML M2 and M1.Least positivity was seen in AML M7 and AML M3

The above table shows that expression of marker HLADR was seen in 71.70% of cases (38/53). Maximum positivity was seen in M7,M1,M2 types of AML. Least positivity was seen in AML M3.

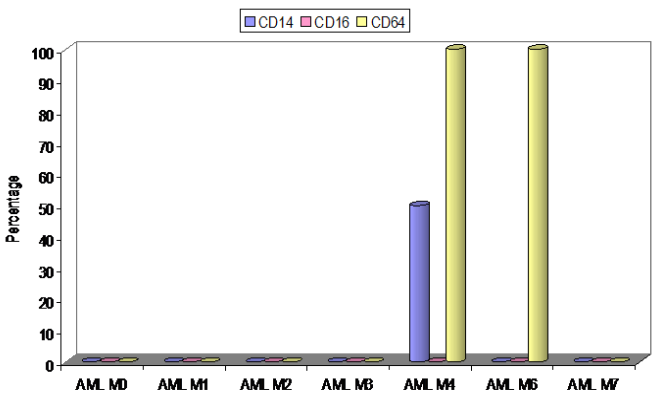
The above table shows that expression of marker MPO was seen in 78.69% of cases (48/61). Maximum positivity was seen in M3 (100% cases), followed by AML M2 and M1. Least positivity was seen in AML M7.

Minimal expression of CD34 and HLADR in AML M3 was found to be statistically significant.

Table 11c: Comparison of Immune Markers positivity in different morphological diagnosis

	M0	M1	M2	M3	M4	M6	M7	Total	²	P
CD14										
<20%	–	1	2	1	2	4	1	11	5.318	0.378
	–		100.00	100.00		100.00	50.00	100.00	100.00	84.62
>20%	–		0	0		0	2	0	0	2

	–	0.00	0.00	0.00	50.00	0.00	0.00	15.38
Total	–	1	2	1	4	4	1	13
CD16								
<20%	–	1	–	1	2	4	1	9
–	–							
	–	100.00	–	100.00	100.00	100.00	100.00	100.00
>20%	–	0	–	0	0	0	0	0
	–	0.00	–	0.00	0.00	0.00	0.00	0.00
Total	–	1	–	1	2	4	1	9
CD64								
<20%	1	1	2	–	0	3	1	8
8.625	0.125							
	100.00	100.00	100.00	–	0.00	75.00	100.00	66.67
>20%	0	0	0	–	3	1	0	4
	0.00	0.00	0.00	–	100.00	100.00	0.00	33.33
Total	1	1	2	–	3	4	1	12



The above table shows that expression of marker CD14 was seen in 15.38% of cases (2/11). Positivity was seen in M4 (50% cases) while all the other subtypes did not show its expression.

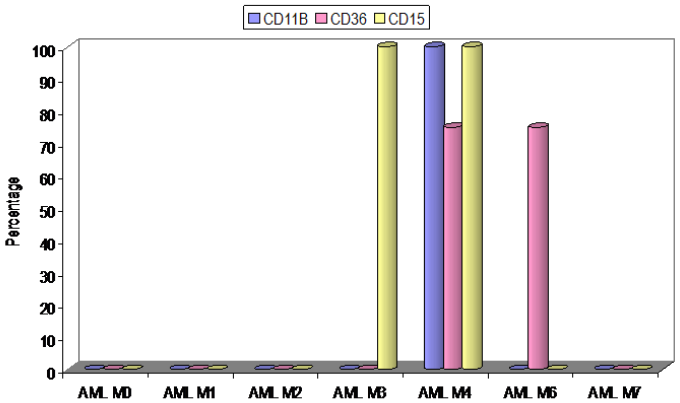
The above table shows that expression of marker CD16 was not seen in any of the 18 cases in which it was interpreted.

Also, the table shows that expression of marker CD64 was seen in 33.33% of cases (4/12). Maximum positivity was seen in M4 and M6 (100% cases) and no expression was seen in other types.

Table 11d: Comparison of Immune Markers positivity in different morphological diagnoses

	M0	M1	M2	M3	M4	M6	M7	Total	^{2}	P
CD36										
<20%	1	–	–	1	1	1	1	5	4.950	0.292
	100.00	–	–		100.00	25.00	25.00	100.00		45.45
>20%	0	–	–		0	3	3	0		6
	0.00	–	–		0.00	75.00	75.00	0.00		54.55
Total	1	–	–		1	4	4	1		11
CD11b										
<20%	–	–	–		–	0	2	–		2
5.000	0.025									
	–	–	–		–	0.00	100.00	–		40.00
>20%	–	–	–		–	3	0	–		3
	–	–	–		–	100.00	0.00	–		60.00
Total	–	–	–		–	3	2	–		5
CD15										
<20%	–	–	–		0	0	–	–		

–	–						
	–	–	–	0.00	0.00	–	–
>20%	–	–	–	1	1	–	–
	–	–	–	100.00	100.00	–	–
Total	–	–	–	1	1	–	–



The table below shows that the expression of marker CD36 was seen in 54.55% of cases (6/11). Positivity was seen in M4 and M6 (75% cases) while all the other subtypes did not show its expression.

The table below shows that the expression of marker CD11b was seen in 60% of cases (3/5). Positivity was seen in M4 (100% cases) while all the other subtypes did not show its expression.

The table below shows that the expression of marker CD15 was seen in AML M3 and M4 cases.

Findings related to expression of immune markers HLADR, MPO, CD117, CD34, CD11B were found to be statistically significant.

Table 11e: Comparison of Immune Markers positivity in different morphological diagnoses

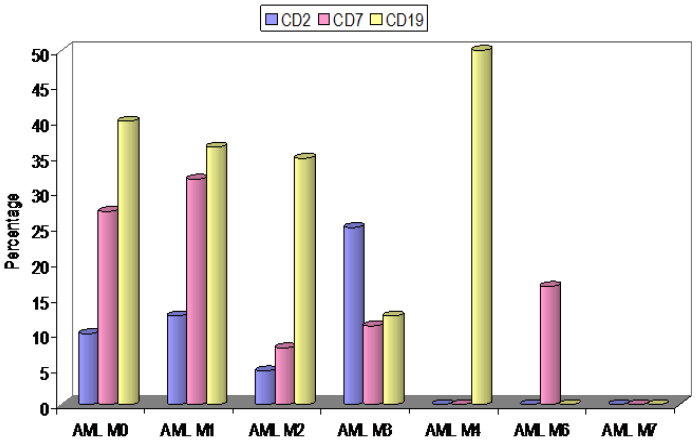
	M0	M1	M2	M3	M4	M6	M7	Total	^{2}	P
CD3										
<20%	5	12	11	5	2	–	–	35		

–	–							
	100.00	100.00	100.00	100.00	100.00	–	–	
100.00								
Total	5	12	11	5	2	–	–	35
CD10								
<20%	11	21	19	8	2	2	–	63
2.235	0.816							
	100.00	100.00	95.00	100.00	100.00	100.00	–	
98.44								
>20%	0	0	1	0	0	0	–	1
	0.00	0.00	5.00	0.00	0.00	0.00	–	
1.56								
Total	11	21	20	8	2	2	–	64
CD11c								
<20%	–	–	–	–	0	–	–	0
–	–							
	–	–	–	–	0.00	–	–	
0.00								
>20%	–	–	–	–	1	–	–	1
	–	–	–	–	100.00	–	–	
100.00								
Total	–	–	–	–	1	–	–	
1								

CD10 was found to be positive in only 5% cases of AML M2 and CD11c in 100% cases of AML M4while CD3 was found in none of the subtypes.

Table 12: Aberrant expression of immune markers in different AML subtypes.

	M0	M1	M2	M3	M4	M6	M7	Total	^{2}	P
CD2										
<20%	9	14	20	3	2	2	–	50	2.336	0.801
	90.00	87.50	95.24	75.00	100.00	100.00	–			90.91
>20%	1	2	1	1	0	0	–	5		
	10.00	12.50	4.76	25.00	0.00	0.00	–			9.09
Total	10	16	21	4	2	2	–	55		
CD7										
<20%	8	15	23	8	3	5	1	63		
6.304	0.390									
	72.73	68.18	92.00	88.89	100.00	83.33	100.00			81.82
>20%	3	7	2	1	0	1	0	14		
	27.27	31.82	8.00	11.11	0.00	16.67	0.00			18.18
Total	11	22	25	9	3	6	1	77		
CD19										
<20%	6	14	15	7	1	5	–	48		
4.651	0.460									
	60.00	63.64	65.22	87.50	50.00	100.00	–			68.57
>20%	4	8	8	1	1	0	–	22		
	40.00	36.36	34.78	12.50	50.00	0.00	–			31.43
Total	10	22	23	8	2	5	–	70		



The following table shows that aberrant expression of marker CD2 was present in 9.09% (5 out of 55 cases) of AML. Maximum positivity was seen in AML M3 (25% cases) followed by AML M1, M0 and M2 and absent in AML M4, M6 and M7.

The table also shows that aberrant expression of marker CD7 was present in 18.18% (14 out of 77 cases) of AML.Maximum positivity was seen in AML M1 (31.82% cases) followed by AML M0, M6 and M2 and absent in AML M4 and M7.

We can also infer from the following table that the aberrant expression of marker CD19 was present in 31.43% (22 out of 70 cases) of AML. Maximum positivity was seen in AML M4 (50% cases) followed by AML M0, M1,M2 and M3 and absent in AML M6 and M7.

Table 13: Comparison of cytogenetics found in different morphological diagnoses

	M0	M1	M2	M3	M4	M6	M7	Total	^{2}	P
t(8;21)										
Negative	5	8	5	7	1	4	30	12.593	0.028	
	100.00	100.00	55.56	100.00	100.00	100.00	88.24			
Positive	0	0	4	0	0	0				4

	0.00	0.00	44.44	0.00	0.00	0.00		11.76
Total	5	8	9	7	1	4		34
1(16;16)								
Negative	5	8	9	7	1	4	–	34
–	–							
	100.00	100.00	100.00	100.00	100.00	100.00	–	100.00
Positive	0	0	0	0	0	0	–	0
	0.00	0.00	0.00	0.00	0.00	0.00	–	0.00
Total	5	8	9	7	1	4	–	34
t(15,17)								
Negative	5	8	9	0	1	4	–	27
34.000	<0.001							
	100.00	100.00	100.00	0.00	100.00	100.00	–	79.41
Positive	0	0	0	7	0	0	–	7
	0.00	0.00	0.00	100.00	0.00	0.00	–	20.59
Total	5	8	9	7	1	4	–	34
Deletion 20/20q								
Negative	–	0	–	–	–	–	–	0
–	–							
	–	0.00	–	–	–	–	–	0.00
Positive	–	1	–	–	–	–	–	1
	–	100.00	–	–	–	–	–	100.00
Total	–	1	–	–	–	–	–	1

Cytogenetics was done in only 35 cases out of 95. Out of three translocations, t (15;17) was found to be the most common. It was detected in 20.59% (7/34) of cases.Infact, all the cases (100%) of

AML M3 were positive for this translocation in which it was done. All the other subtypes of AML were negative for t(15;17) in the cases in which it was performed.

The translocation t(8;21) was found to be the next most common.It was detected in 11.76% (4/34) of cases.Infact, 4 out of 9 cases (44.44%) of AML M2 were positive for this translocation. All the other subtypes of AML were negative for t(8;21) in the cases in which it was performed. These findings were found to be statistically significant.

The translocation t(16;16) was not detected in any of the AML subtypes, in the cases in which it was done.

DISCUSSION

A total of 95 newly diagnosed cases of acute myeloid leukemia were evaluated in our study. Their peripheral blood samples and bone marrow aspirates were studied for the morphology, cytochemical staining and immunophenotypic profile of the blast cells.

In our study population (ranging 7 months to 80 yrs), 65.26% (62/95) patients were less than 16 yrs of age while the rest, 34.74% (33/95) patients were

> or = 16 yrs of age. Out of which, 21.05% were between 16 to 45 years of age and 13.68% were above 45 years. The mean age at the time of diagnosis was 18.76 yrs. This division holds importance because it has been seen that AML has a worse prognosis in adults aged >45 years. We know that AML is more common in adults, contrary to our findings here. Also, in other studies conducted such as Ansari et al (in 2003) it was found that seventy-six percent of their cases were adults. In their study, the age of the patients ranged from one year to 78 years with a median age of 27.2 years(46). The difference in age distribution may be attributed to the more paediatric cases visiting our hospital.

The commonest FAB subtype was Acute Myeloid Leukemia With Maturation (31/95; 32.63% cases) while the least common was Acute Myelomonocytic Leukemia (1/95;1.05% cases). The order was Acute Myeloid Leukemia with maturation□M1□ M3□M0□M6□M4□M7□M5. Acute Myeloid Leukemia Without Maturation and Acute Myeloid Leukemia with maturation both are the most common subtypes in the pediatric population while Acute Myelomonocytic Leukemia is the least common. Also, Acute Myeloid Leukemia with maturation is the most common subtype in the adult population while Acute Myelomonocytic Leukemia, AML M5 and AML M7 are the less common subtypes. Other studies have also observed a higher frequency of Acute Myeloid Leukemia with maturation in both pediatric and adult population from the same institution. (46) The proportion of Acute Monocytic Leukemia in our series was 4.21 % only. Roberts et al have reported a frequency of 40% in case ofAcute Monocytic Leukemia.[53] The incidence of

Acute Promyelocytic Leukemia in our study was 12.63%. Incidence of AML-M6 was 8.42%, more than 3-5% quoted in the literature. (77,78,79)

Most common morphological diagnosis was Acute Myeloid Leukemia with maturation (32.63%) followed by Acute Myeloid Leukemia without maturation (27.37%), Acute Promyelocytic Leukemia (12.63%),Acute Myeloid Leukemia with minimal differentiation (11.58%), AML-M6 (8.42%),Acute Monocytic Leukemia (4.21%), AML-M7 (2.11%) and least common was AML-M5 (1.05%).

Comparison of the percentage of subtypes of AML in different studies

Author	Total cases	M0	M1	M2	M3	M4	M5	M6	M7
Ansari et al (46)	260	--	16	32	5	20	19	1.6	0
Advani et al		--	--	34	5.4	3.5	20	1.6	--
Wintrobe (62)		---	10-20	---	7-8	5-10	2-10	2-4	0.6-10
Ihsan (55)	106	27.9	21.2	13.5	13	12.5	5.8	--	5.8
Our study	95	11.58	27.37	32.63	12.63	4.21	1.05	8.42	2.11

The minimum age was seen in Acute Megakaryoblastic Leukemia while the maximum was seen in Acute Myeloid Leukemia with maturation subtype. The highest mean is seen in the Acute Myelomonocytic Leukemia subtype.

There were 54 males and 41 females with a male to female ratio of 1.3:1. In a similar study by Ansari et al, the ratio obtained was 2.56:1.(46) Also, this ratio was 1.26:1 in a study by S Gujral et al(33). The most and least common FAB type in females are Acute Myeloid Leukemia Without Maturation and Acute Monocytic Leukemia respectively. While in males it is the Acute Myeloid Leukemia With Maturation subtype which is the most common and Acute Monocytic Leukemia and Acute Megakaryoblastic Leukemia are the less common types.

Amongst the clinical findings, the most common was fever (72.63%) followed by fatigue (55.79%), weight loss (48.42%) and infection, including diarrhea, respiratory tract infections(48.42%), the less common ones were bleeding(40%), lymphadenopathy(26.32%), bone pain(20.21%),

hepatomegaly(17.89%) and splenomegaly(14.74%) while the least common was abnormal mass manifesting as orbital swelling in all the cases (3.16%). However, a higher percentage (approximately 75%) has been reported in the literature(73,74) In a similar study by Ansari et al (46) the majority of their patients (82%) presented with pallor and weakness. Lymphadenopathy was fairly common and seen in 94 patients (36.2%). Cervical lymph nodes were most often involved followed by the axillary nodes. Hepatosplenomegaly was seen in 68 patients (26.2%). Fever was a prominent most common presenting symptom seen in 72.63% (69/95) of the patients.

In our study, the proportion of patients presenting with fever was higher in Acute Promyelocytic Leukemia (83.33%), Acute Myeloid Leukemia without Maturation (80.77%), Acute Myeloid Leukemia with Maturation (77.42%) and Acute Myelomonocytic Leukemia (75.00%) as compared to patients with other morphological diagnosis (0 to 54.55%).

Splenomegaly was most common in AML M1 subtype followed by AML M0 and least common in AML M4, M5 and M7. Hepatomegaly was most common in AML M2 and M3 subtype and least common in AML M4 and M5. Lymphadenopathy was most common in AML M0 subtype followed by AML M2 and M1 and least common in AML M5 and M7.

Of all subtypes, acute promyelocytic leukemia cases had a significantly (p=0.041%) higher proportion (83.33%) of patients with bleeding symptoms while the rest 16.66% cases did not manifest with bleeding. It was seen in the mucosa and skin. Ansari et al in 2003 reported that bleeding was present in 57 patients (21.9%) out of 260 cases. It was most common in the AML-M3 and M5b categories and seen in the mucosa and skin. AML M3 was followed by AML M1 for having bleeding manifestation. The mean platelet value was least in AML M3. However, it was low in other AML subtypes as well, but they showed much lesser bleeding manifestation. Thus, depicting the possibility of other causes for bleeding in AML M3 which is well supported by literature.(15)

Abnormal mass (i.e. orbital swelling) was found in only 3 (3.16%) patients. Abnormal mass was observed in 2 (6.45%) patients of Acute Myeloid Leukemia with maturation and 1 (25.0%) patients

of Acute Monocytic Leukemia. Thus, these cases presented with extramedullary leukemia with orbital masses.

Anemia was seen to be the most common finding at the time of presentation as 95.79% (91/95) of the patients had hemoglobin levels less than 10gm%. The Haemoglobin levels ranged from 2.6 to 12.6 gm% with a mean value of 6.03 gm% ± 1.89 (SD). The lowest as well as the highest values were found in Acute Myeloid Leukemia with maturation subtype. Acute Monocytic Leukemia had the lowest mean value while Acute Myeloid Leukemia with Minimal Differentiation had the highest mean value.

The total leukocyte count ranged from 1100 cells/cu.mm to 4,10,000 cells/cu.mm. The TLC had mean value 37,872 cell/cu.mm ± 57400 cells/cu.mm (SD). The lowest value was seen in acute myeloid leukemia with maturation while the highest value was found in acute myeloid leukemia with minimal differentiation subtype. Acute monocytic leukemia had the lowest mean value while the acute myeloid leukemia with minimal differentiation had the highest mean value. In the literature, however, AML M4 is quoted to have a higher TLC count commonly(62). Most of the patients (67.37%) presented with leukocytosis (Total leukocyte count >11000/cumm). As 32.63% of the patients presented with low to normal leukocyte count, it was inferred that thorough examination of peripheral blood smears was necessary in routine hematological examination to prevent missing out on early diagnosis of leukemia cases. Ansari et al did a similar study and found the following findings. The WBC count was highest in case of Acute Myeloid Leukemia without maturation with a mean of 97x109/L followed by the Acute Monocytic Leukemia and Acute Monocytic Leukemia subtypes (80-90 x109/L). The lowest WBC counts were observed in the Acute Promyelocytic Leukemia category as 10 x109/L.

Leucocytopenia (<11,000/ cu.mm) was found in 31 (32.63%) patients of the study population. Proportion of patients with leucocytopenia was found in higher proportion of patients of AML-M3 (50.0%), AML-M7 (50.0%) and AML-M1 (42.31%) as compared to patients with other morphological diagnosis.

Thrombocytopenia was seen in 91.58% (87/95) patients with platelet count <1.0lac/cumm. The platelet count ranged from

1100 cells/cu.mm to 4,10,000 cells/cu.mm. The mean value was 39643l/cu.mm ± 41903 cells/cu.mm (SD). The lowest value was seen in acute myeloid leukemia with maturation and AML M1 while the highest value was found in acute myeloid leukemia with maturation subtype. Acute promyelocytic leukemia had the lowest mean value while AML M7 had the highest mean value. AML M7 is also quoted in the literature to have higher platelet counts.(62)All the patients of acute myeloid leukemia with minimal differentiation, acute promyelocytic Leukemia, Acute Myelomonocytic Leukemia, and Acute Monocytic Leukemia showed thrombocytopenia. Though proportion of patients with platelet count >1 lac was higher in AML-M7 (50.0%) and AML-M6 (25.00%) as compared to patients with other morphological diagnosis but this difference was not found to be statistically significant.

PBS-blast count (<20%) was found in 27 (23.16%) patients. Thus, 23.16% cases were in aleukemic phase. Proportion of patients with PBS-blast count >20% was highest in acute myeloid leukemia with minimal differentiation and acute promyelocytic leukemia and significantly lower in Acute Monocytic Leukemia (25.0%) as compared to patients of other morphological diagnosis. The single patient diagnosed as AML-M5 had PBS-blast count <20% (ie was in aleukemic phase).AML M5, M4 and M7 had maximum number of aleukemic cases.

Comparison between haematological parameters in our study and Ihsan et al

Hb(mean) g/dl

Our Study Hb(mean) g/dl

Ihsan et al TLC(mean)
(cells/cumm)

Our Study TLC(mean)
(cells/cumm)

Ihsan et al Platelet (mean)(per cumm)

Our Study	Platelet (mean)(per cumm)					
Ihsan et al						
AML MO	6.8	8.4	68318	39700	26182	94000
AML M1	5.7	9.0	31242	52500	42150	83200
AML M2	6.14	7.8	45655	35000	44116	39800
AML M3	6.18	7.3	22975	25000	23450	53700
AML M4	4.73	8.1	46300	30100	35250	74900
AML M5	4.0	9.1	11600	62200	35000	63800
AML M6	6.23		14525		53625	
AML M7	6.35	6.9	15000	73500	64100	50800

Number of bone marrow blast cells were found to be significantly lower in patients diagnosed as AML M4 (38.00+17.26), AML M5 (58.00) and AML M6 (57.50+50.20) as compared to patients with other diagnosis. Highest blast count was seen in AML M2 followed by AML 3 and AML M1.The mean value of blast count was 71.18 ± 20.65.

Author	Auer rods seen
Neelam et al	40% casesof AML
Our study	54.7% cases of AML

We studied the details of nucleus in blast cells in the peripheral blood smears and bone marrow smears. The nucleus was rounded in majority of the blast cells in acute myeloid leukaemia while in acute monocytic leukaemia, the nucleus had an irregular shape due to indenta¬tions and lobulation. Nuclear chromatin was fine in blast cells of acute myeloid leukaemia. Similar findings were seen in the study by Ihsan M. Elhadi et al.(55)

Author	**Bone marrow Blasts**	**Subtype with highest blast count**	**Subtype with lowest blast count**
Advani et al	57.6% (34- 96)	Acute Myeloid Leukemia without maturation	M4
Our study	71.18(21 – 98)	M3	M4

In each case the slides were subsequently stained to assess their reactivity for myeloperoxidase (MPO) stain. It was seen that 64/

84 (76.19%) cases showed positive cytochemistry for MPO out of 84 cases diagnosed as AML M1, M2, M3 and M4 and excluding AML M5, M6 and M7 cases because they were predominantly negative correlating with the literature. 65.38% cases of AML M1 were positive for MPO. Maximum positivity was seen in AML M3 (100%) category, followed by AML M2(90.32%) while minimum in AML M5 and M7(0%). Statistically, the positivity of MPO in identifying the myeloid lineage was found to be significant. ($p<0.001$)

Amongst the undifferentiated leukemia cases as reported on morphological evaluation, 3 cases out of 6 showed positivity for MPO staining. All these six cases, when assessed for immunophenotyping diagnosis, showed expression of myeloid markers (CD13, CD33, CD117), thereby, confirming the myeloid lineage.

Concordance between Morphological and Immunophenotypic Diagnosis

Morphological Diagnosis	No. of cases	Immunophenotypic Diagnosis
AML	89	95
Undifferentiated	6	
	95	95

Agreement between Morphological against Immunophenotypic diagnosis for AML cases: 95/95 = 100%.

We compared our finding with other studies:

Year	Author	Total no. of cases studied	% MPO concordanceAML
2010	Mukda et al.(52)	114	89.6
2009	S Gujral et al(33)	964	75
2013	Belurkar et al.(44)	50	91.6
2014	Our study	95	69.47

With the results obtained, we reached to the conclusion that cytochemistry in our cases helped in establishing the lineage correctly in 76.19% cases. MPO is sensitive as well as specific for identifying precursors of myeloid lineage. Even though MPO enabled us to assign lineage in the 3 out of 6 cases of undifferentiated leukemia as categorized on morphology, the findings need to be confirmed with immunophenotyping. Nevertheless, the importance of MPO stain

cannot be subdued especially for centres where flowcytometry is yet to become a routine practice.

The diagnosis thus made on morphological and cytochemical grounds was further subjected to immunophenotypic analysis for confirmation.

When compared with previous studies, similar results were observed:

Year	Author	Total no. of cases studied	No. of AML cases with concordant diagnosis	Number of AML cases Percentage	
1998	Kheiri et al. (10)	93	37	33/37	89.1%
2004	Kresno et al. (48)	225	115	111/115	96.52%
2006	Qadir et al. (50)	646	525	522/525	99.43
2013	Belurkar et al(44)	50	12	12/12	100%
2015	Our study	95	95	95/95	100%

CD33 was the myeloid marker that was most commonly present in all the AML subtypes i.e.93.98% cases. CD13 was the next most common marker present in all the AML subtypes i.e.85.54% cases. In our study, the expression of marker CD13 was seen in 85.54% of cases (71/83). The expression of marker CD33 was seen in 93.98% of cases (78/83). Maximum positivity of CD33 was seen in M3,M4,M6 and M7 (100% cases) of AML followed by AML M2, M1 and M0. In a study by Ihsan et al, CD33 was found to have higher positivity among AML-M4 and AML-M5 with mean positivity of 75.9% and 76.6% respectively.

In our study, CD117 had maximum positivity seen in M6 and M7 (100% cases) of AML followed by AML M2, M1, M0, M4 and M3.Expression of marker MPO was seen in 78.69% of cases (48/61). Maximum positivity was seen in M3 (100%cases), followed by AML M2 and M1. Least positivity was seen in AML M7. Expression of marker CD34 was seen in 74.36% of cases (58/78). Maximum positivity was seen in M0 (100% cases) of AML followed by AML M2 and M1.Least positivity was seen in AML M7 and AML M3. In our study, the positivity of the stem cell marker CD34 was in the range of 0% to 99% among the various subtypes. Expression of marker HLADR was seen in 71.70% of cases (38/53). Maximum positivity was seen in M7,M1,M2 types of AML. Least positivity

was seen in AML M3. HLA-DR was in the range of 0% to 99.6%.

The positivity of the marker MPO was in the range of 0% to 99.3% among the various subtypes. 62.5% cases of AML M0 were positive for MPO on flowcytometry while on cytochemical staining by MPO, only 36.36% cases of AML M0 were positive. Hence, flowcytometry is a more sensitive method for MPO identification.

Acute Myeloid Leukemia With Minimal Differentiation, Acute Myeloid Leukemia without maturation and Acute Myeloid Leukemia with maturation cases were positive for CD34, CD13, CD33, CD117 and HLADR. Cases of acute promyelocytic leukemia showed strong MPO positivity. CD34 and HLA-DR expression was absent in most of the cases of promyelocytic leukemias. . This was supported in a study by Cesare Guglielmi et al in 199855 who had correlated the immunophenotype of adult and childhood acute promyelocytic leukaemia with morphology. They confirmed in APL an immunophenotype characterized by frequent expression of CD13, CD33 and CD9 and rare expression of HLA-DR, CD10, CD7 and CD11b. Similar findings were seen in the study by Elisabeth Paietta(38) and Bakke et al (76)

Although the diagnosis could not be supported by cytochemical staining, flowcytometry showed positivity for CD14 and CD11b along with other myeloid markers, thus confirming the finding of Acute Myelomonocytic Leukemia (Table 10). CD14 expression ranged between 11% to 87% and the highest was seen in the Acute Myelomonocytic Leukemia subtype.

Immunophenotyping could not be done on our only case of Acute Myelomonocytic Leukemia. Flowcytometry could not to be of any further use for our cases of AML6 and AML M7 as we did not have the specific markers for erythroid and megakaryocytic lineage.

Comparison of immune markers positivity in our study

	CD34	HLADR	CD13	CD33	CD117	MPO	CD7	CD19
AMLM0	100	80	81.82	90.91	70	62.50	27.27	40
AMLM1	81.82	90.91	79.17	91.67	86.96	88.89	31.82	36.36
AMLM2	83.33	82.35	85.19	92.59	92.59	94.12	8	34.78
AMLM3	20	20	90	100	44.44	100	11.11	12.50
AMLM4	75	75	100	100	50	66.67	0	50

AMLM5								
AMLM6	66.67	80	100	100	100	0	16.67	0
AMLM7	0	100	100	100	100	78.69	0	0
Mean(%) positivity		74.36	71.70	85.54	93.98	81.25	78.69	
18.18	31.43							

Comparison of immune markers positivity in study by Ihsan et al

	CD34	HLA	CD45	CD13	CD33	CD117	CD19	CD7
AML-M0	68.63%	47.14%	89.62%	56.81%	38.42%	61.50%	12.87%	31.89%
AML-M1	57.85%	48.80%	90.06%	51.12%	45.22%	55.81%	11.58%	19.94%
AML-M2	62.79%	51.99%	88.32%	49.85%	53.84%	56.68%	9.48%	33.37%
AML-M3	17.32%	13.13%	91.08%	58.94%	54.29%	22.49%	14.08%	22.26%
AML-M3V	17.32%	24.47%	96.87%	47.70%	67.50%	53.90%	1.52%	27.15%
AML-M4	41.64%	41.95%	93.86%	54.86%	65.98%	35.00%	2.88%	23.36%
AML-M5	23.38%	64.71%	94.88%	38.34%	76.64%	13.10%	0.84%	16.41%
AML-M7	32.35%	25.34%	91.92%	33.72%	56.12%	21.04%	7.72%	38.40%
Total	50.36%	42.87%	90.81%	51.73%	50.35%	49.44%	9.58%	27.37%

Similar study was done in Tata Memorial Hospital by Ansari et al in 2003 on 260 cases of AML, which showed the positivity of the stem cell marker CD34. CD34 was in the range of 18.5% to 66.7% among the various subtypes. The highest positivity was seen in the AMLM0 and AML M6. HLA-DR positivity was highest in case of AML M2 and was less in the AML M0 and M4 subtypes. CD34 and HLA-DR expression was absent in all cases of promyelocytic leukemias. CD33 was the myeloid marker that was most commonly present in all the AML subtypes. CD13 was the next most commonly expressed antigen showing 100% positivity in the AML M3, M4 and M6 categories and ranging from 80-90% in the other subtypes. CD14 and CD36 positivity was more commonly associated with the monocytic leukemias. CD14 expression ranged between 3.7% to 44.4% and the highest was seen in the AML M4. CD36 expression was highest in the AML M5 and less in AML M4 categories. In their study, expression of lymphoid antigens was seen in 15% cases of AML. CD7 was the most commonly expressed lymphoid antigen (11%) .

Author	CD13(%positivity)	CD33(%)	CD117(%)
S Gujral et al	92	88	73
Ihsan et al	51.5	49.8	
Our study	85.54	93.98	34

Author	HLA-DR	CD34	CD117	CD13			
S Gujral et(33) al(2009)		95	95	51	2	%	Acute Promyelocytic Leukemia cases Negative
Our study	80	80	55.56	10	% Acute Promyelocytic Leukemia cases Negative		

Also flowcytometry helped us to study the aberrant markers present in leukemia cases. Aberrant markers are the unusual immunophenotypes. These are described as cross lineage antigen expression, expression of markers that are asynchronous with the level of maturity and over or underexpression of antigens. In our study, 63 out of 95 cases (66.3%) showed expression of expected antigens only. The remaining 33.68% (32/95) showed presence of aberrant markers, most common being CD19 (31.43%) followed by CD7(18.18%) and CD2(9.09%). The diagnosis of mixed phenotypic leukemia was ruled out as the WHO criteria were not satisfied(Annexure 1).

Despite the presence of aberrant markers, myeloid origin of blasts is not doubtful because of additional confirmation using MPO (cytochemical and immunophenotyping MPO).

Year	Author	No. of AML cases	Cases showing aberrant markers
2010	Mukda et al. (52)	30	14 (46.67%)
2014	Mehdi Jaredi et al(79)	56	32 (57.1%)
2015	Our study	95	32 (33.68%)

Year	Author	No. of AML cases	CD19 +	CD7+	CD2+	
2010	Mukda et al. (52)	30	64.2%	35.71%	----	
2011	Ihsan et al(55)	106	13.6	45.1		
2007	Abdelhaleem et al(75)	59	22%	25.8%	15.5%	
2015	Our study	95	31.43%	18.18%	9.09%	

Year	Author	No. of AML cases	Cases showing aberrant markers							
CD	Acute Myeloid Leukemia Without Maturation		Acute Myeloid Leukemia With Maturation		Acute Promyelocytic Leukemia		Acute Myelomonocytic Leukemia		Acute Monocytic Leukemia	
2014	Mehdi Jaredi et al	56	32 (57.1%)		CD2	58.35	21.40	33.3	------	20
			CD7	72.7	28.5	-----	------	-----		
2015	Our study	95	32(33.68%)		CD2	12.5	4.76	25	-----	-----
			CD7	31.82	8.0	11.11	----	------		

Also, in a study by Ihsan et al, CD7 was mostly expressed in AML-M2 and AML-M3 (75%) and least in AML-M5, while CD19 was only expressed in cases of AML-M0 and AML-M7.

Cytogenetics was done in only 35 cases out of 95. Out of three translocations, t(15;17) was found to be the most common. It was detected in 20.59% (7/34) of cases. Infact, all the cases (100%) of AML M3 were positive for this translocation in which it was done. All the other subtypes of AML were negative for t(15;17) in the cases in which it was performed.

The translocation t(8;21) was found to be the next most common.It was detected in 11.76% (4/34) of cases. Infact, 4 out of 9 cases (44.44%) of AML M2 were positive for this translocation. All the other subtypes of AML were negative for t(8;21) in the cases in which it was performed. These findings were found to be statistically significant well correlated with the literature.(62)

The translocation t(16;16) was not detected in any of the AML subtypes, in the cases in which it was done.

The present study reemphasized the importance of morphological diagnosis of acute leukemia by scrupulous examination of peripheral blood and bone marrow aspirate smears even in the age of immunophenotypic diagnosis. In under-resourced hematology laboratories there is usually a missing step in the battery of required investigations. Moreover, when some of the advanced diagnostic instruments can be found then the problem

of chronic inadequate and irregular supply of kits and services would supervene. Therefore, the laboratory diagnosis would mostly depend on the more basic, but consistently available and well controlled, laboratory techniques that should at least include complete blood count (CBC) and peripheral blood morphology, after which a bone marrow study with aspirate will follow.(41)

CONCLUSION

Our study was conducted at the Department of Pathology, King George Medical University, Lucknow. The study was prospectiveand retrospective descriptive type. We evaluated a total of 95 newly diagnosed cases of acute myeloid leukemia. We did a complete blood count (CBC) and peripheral blood morphology, after which a bone marrow aspirate was done. Bone marrow aspiration was subjected to morphological, cytochemical (myeloperoxidase staining) and immunophenotyping diagnosis.

Following conclusions were made,

- In our study population (ranging 7 months to 80 yrs), majority of the population comprised of paediatric patients. This could be attributed to the referral bias due to the more paediatric patients visiting our hospital. Paediatric patients comprised a good percentage of AML cases. The minimum age was seen in AML M7 subtype (7 months).
- Number of male patients was slightly more than the female patients.
- Overall non specific bone marrow failure symptoms and signs were seen. Overall most common symptom was fever followed by fatigue, weight loss and infection, the less common ones were bleeding, lymphadenopathy, bone pain, hepatomegaly and splenomegaly while the least common was abnormal mass. Bleeding was significantly found in cases of acute promyelocytic leukemia. However, they were not very helpful in differentiating the AML subtypes.
- The most common subtype of AML was acute myeloid leukemia with maturation .This finding well corroborates with the reported literature.
- Acute erythroid leukemia cases were diagnosed slightly more in number as compared to the previously reported literature.
- The total leukocyte count was raised in majority of cases. However, it was reduced in 1/3 rd of the cases.
- Peripheral blood blast count was >20% in majority cases.

However, approximately 1/4 th (23.16%) aleukemic cases were seen. Thus, highlighting the significance of careful morphological examination of the PBS smears.

• Thrombocytopenia was seen in majority patients. However, normal platelet counts were found in 1/12 th cases.

• The evaluation of morphological features of myeloid blasts was helpful in categorising subtypes of AML eg. Monocytoid blasts in AML 4 and AML5 and pleomorphic lobulated in AML M3.

• Auer rods were significant in identifying AML M1, M2 and M3 subtypes.

• There was 100% concordance between morphological and Immunophenotypic diagnosis and 76.19% concordance between cytochemical and Immunophenotypic Diagnosis.

• CD33 was the myeloid marker that was most commonly present in all the AML subtypes i.e.93.98% cases. CD13 was the next most common marker present in all the AML subtypes i.e.85.54% cases.

• AML M0, M1 and M2 cases were positive for CD34, CD13, CD33, CD117, MPO and HLADR.

• Cases of acute promyelocytic leukemia showed strong MPO positivity. Immunophenotyping showed CD34 -- and HLADR -- in AML M3 cases along with strong positivity for CD13 and CD33.

• Flowcytometry showed positivity for CD14 and CD11b along with other myeloid markers, thus confirming the finding of AML M4.

• As we did not have the specific markers for erythroid and megakaryocytic lineage, flowcytometry could not to be of any further use for our cases of AML6 and AML M7.

• Approximately, 1/3rd cases showed presence of aberrant markers, most common being CD19 followed by CD7 and CD2. Therefore, a extended panel as recommended by WHO is advised to rule out mixed phenotypic acute leukemia.

• As per cytogenetic data collected in the various AML subtypes, t(15;17) was most commonly seen in Acute promyelocytic leukemia subtype and t(8;21) in Acute myeloid leukemia with maturation.

Thus, complete and meticulous examination of peripheral blood and bone marrow aspirate smears helps in making the diagnosis in majority of the cases of acute leukemia. In cases where morphological features overlap or are inadequate for establishing a definitive lineage, cytochemistry comes into use. Myeloperoxidase is easily done, cheap and sensitive for identification of myeloid series of cells.

However, definitive diagnosis can only be made using flowcytometry as cytochemical staining may be equivocal in few cases.

Flowcytometry can also help in identifying expression of aberrant markers and making the diagnosis in case of acute leukemia of ambiguous lineage.

Thus concluding, our study emphasized the significance of morphological diagnosis of acute leukemia by scrupulous examination of peripheral blood and bone marrow aspirate smears even in the era of immunophenotypic diagnosis.Also, we cannot understate the contribution of cytochemical studies, as they provide a cheaper alternative, in centres where flowcytometry cannot be done because of financial restrictions. But, again immunophenotyping has become indispensable in the present scenario and is a boon to the diagnosis of acute myeloid leukemia.

REFERENCES

1. Bob Lowenberg, James R. Downing, Alan Burnett. Acute Myeloid Leukemia. N Engl J Med 1999;341:1051-1062.

2. A L Thornbun. Alfred François Donné, 1801-1878, discoverer of Trichomonas vaginalis and of leukaemia. British Journal of Venereal Diseases 1974 Oct; 50(5): 377–380.

3. Kampen KR. The discovery and early understanding of leukemia. Leuk Res 2012 Jan;36(1):6-13.

4. Ekchol Mukda, Ketsarin Pintaraks, Patcharee Komvilaisak et al. Cytochemistry and Multi-color Flow Cytometric Immunophenotype for Diagnosis of Childhood Acute Leukemia. Journal of Hematology and Transfusion Medicine January-March 2011;vol 21:23-31.

5. Sushma Belurkar, Himabindu Mantravadi, Chethan Manohar et al. Correlation of morphologic and cytochemical diagnosis with flowcytometric analysis in acute leukemia. Journal of Cancer Research and Therapeutics January-March 2013;Vol 9(1):71-79.

6. Vardiman JW, Thiele J, Arber DA et al. Blood.The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood 2009 Jul 30;114(5):937-51.

7. Knapp W (ed) Leukocyte typing IV. White cell differentiation antigens. The Journal of Immunology.Oxford: Oxford Univ Press 1989.

8. Borowitz MJ, Guenther KL, Shults KE, Stelzer GT. Immunophenotyping of acute leukemia by flow cytometric analysis: Use of CD45 and right-angle light scatter to gate on leukemic blasts in three-color analysis. Am J Clin Pathol 1993;100:534.

9. F Lacombe, F Durrieu, A Briais, P Dumain, F Belloc, E Bascans, M R Boisseau, Ph Bernard. Flow cytometry CD45 gating for immunophenotyping of acute myeloid leukemia, Leukemia November 1997;11(11):1878-1886.

10. Samir A. Kheiri, Thomas MacKerrell, Vincent R. Bonagura et al. Flowcytometry with or without cytochemistry for the diagnosis of acute leukemias? Cytometry (Communications in Clinical Cytometry) 1998; 34:82–86.

11. GJ Schiller, Clinical and biological features of favourable - risk acute myelogenous leukemia – is favourable – risk AML really Favourable? Leukemia (2005); 19:326-328.

12. Barbara Deschler, Michael Lubbert, Acute myeloid leukemia: Epidemiology and etiology, American Cancer Society, 2006.

13. Ashkan Emadi, Maria R . Baer. Acute Myeloid Leukemia in adults. John P Greer, Daniel A Arbe, Bertil Glader et al. Wintrobe's clinical hematology,13th edition. Lippincott Williams & Wilkins; 1593,94

14. Gordon J. Piller. Leukaemia – a brief historical review from ancient times to 1950. British Journal of Haematology February 2001;Volume 112(Issue 2): pages 282–292,

15. Jeffrey E Lancet, Peter Maslak, Steven L. Soignet. Acute Promyelocytic Leukemia. John P Greer, Daniel A Arbe, Bertil Glader et al. Wintrobe's clinical hematology,13th edition. Lippincott Williams & Wilkins;1656

16. Taken from http://en.wikipedia.org/wiki/Leukemia

17. Ehrlich, P Farbenanalytische untersuchungen zur histologie und klinick des blutes (Berlin: Hirschwald, 1891).

18. Taken from http://en.wikipedia.org/wiki/Leukemia

19. Taken from http://en.wikipedia.org/wiki/Myeloperoxidase

20. Kaplow SL. Simplified myeloperoxidase stain using benzidine dihydrochloride. Blood 1965;26:215.

21. Graham RC, Karnovsky MJ. The early stage of absorption of injected horseradish peroxidase in the proximal tubule of mouse kidney; Ultrastructural cytochemistry by a new technique. J Histochem Cytochem 1966; 14:291.

22. Leonard S. Kaplo. Brief Report: Simplified Myeloperoxidase Stain Using Benzidine Dihydrochloride August 1,1965; Blood: 26 (2).

23. Robert A. Clark. Peroxidases: A Historical Overview of Milestones in Research on Myeloperoxidase. The Peroxidase Multigene Family of Enzymes. Springer Berlin Heidelberg 2000;1-10.

24. Batra NR, Deshpande C K, Mehta B C et al. Cytochemical studies in leukaemias. J Postgrad Med 1978; 24:226-230.

25. Peter B. Neame, Praniti Soamboonsrup, George P. Browman et al. Classifying Acute Leukemia by Immunophenotyping: A Combined FAB-Immunologic Classification of AML.Blood (December), 1986;vol 68(6): 1355-1362.

26. Bennett JM, Catovsky D, Daniel MT et al. Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. Br J Haematol 1976 Aug; 33(4):451-8.

27. John M. Peters and M. Qasim Ansari. Multiparameter Flow Cytometry

in the Diagnosis and Management of Acute Leukemia. Archives of Pathology & Laboratory Medicine January 2011;135(1): pp. 44-54.

28. Fulwyler MJ. Electronic separation of biological cells by volume. Science 1965; 150 (698): 910–911.

29. Taken from en.wikipedia.org/wiki/Flow_cytometry

30. Herzenberg LA, Parks D, Sahaf B et al. The history and future of the fluorescence activated cell sorter and flow cytometry: a view from Stanford. Clin Chem 2002 Oct;48(10):1819-27.

31. Griffin JD, Mayer RJ, Weinstein HJ, Rosenthal DS, Coral FS, Beveridge RP et al. Surface Marker Analysis of Acute Myeloblastic Leukemia: Identification of Differentiation-Associated Phenotypes. Blood 1983(Sept.); 62(3): 557-563.

32. Tomonaga M, Yoshida Y, Tagawa M et al. Cytochemistry of Acute Promyelocytic Leukemia (M3): Leukemic Promyelocytes Exhibit Heterogeneous Patterns in Cellular Differentiation. Blood 1985(Aug); 66(2):350-357.

33. S. Gujral et al. Immunophenotypic profile of acute leukemia: Critical analysis and insights gained at a tertiary care center in India. Cytometry Part B: Clinical Cytometry 2009 May; Volume 76B(Issue 3): pages 199–205

34. Bloomfield CD, Brunning RD. The Revised French-American-British Classification of Acute Myeloid Leukemia: Is New Better? Annals of Internal Medicine 1985; 103(4).

35. Bennett et al. Proposal for the recognition of minimally differentiated acute myeloid leukaemia (AML-M0). Br J Haematology 1991; 78:325-329.

36. Kumar Abbas, Fausto, Aster. Robbins and Cotran Pathological Basis of Disease. Diseases of White Blood Cells, Lymph nodes, spleen and thymus, 8th edition; 589

37. Browman GP, Neame PB, and Soamboonsrup P. The Contribution of Cytochemistry and Immunophenotyping to the Reproducibility of the FAB Classification in Acute Leukemia. Blood 1986; 68(4): 900-905.

38. Elisabeth Paietta et al. Expression of cell-surface antigens in acute promyelocytic leukaemia. Clinical Hematology September, 2003;16(3);369-85

39. F Lacombe, F Durrieu, A Briais et al. Flow cytometry CD45 gating for immunophenotyping of acute myeloid leukemia. Leukemia 1997; 11: 1878–1886.

40. Head DR. Savage RA, Cerezo L et al: Reproducibility of the French-American-British classification of acute leukemia: The Southwest Oncology Group experience. Am J Hematol 1985;18:45.

41. Abbas H. Abdulsalam. Diagnosis of Acute Leukemia in Under-Resourced Laboratories. December 22, 2011

42. Kheiri SA, MacKerrell T, Bonagura VR et al. Flow Cytometry With or Without Cytochemistry for the Diagnosis of Acute Leukemias?. Cytometry (Communications in Clinical Cytometry) 1998; 34:82–86.

43. James W. Vardiman, Nancy Lee Harris et al. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood 2002;100:2292-2302.

44. Sushma Belurkar, Himabindu Mantravadi, Chethan Manohar et al.Correlation of morphologic and cytochemical diagnosis with flowcytometric analysis in acute leukemia. Journal of Cancer research and Therapeutics 2013; Volume 9: 71-79.

45. Jine Zheng, Xingbing Wang, Yu Hu et al. A Correlation Study of Immunophenotypic, Cytogenetic, and Clinical Features of 180 AML Patients in China. Cytometry Part B (Clinical Cytometry) 74B:25–29 (2008).

46. R Ansari, Y Badrinath, AB Chougule et al. Haematologic and immunophenotypic profile of acute myeloid leukemia : an experience of Tata Memorial Hospital Haematopathology, Laboratory, Department of Medical Oncology, Tata Memorial Hospital, Mumbai, India. Indian Journal of Cancer Vol. 40,(2), April-June, 2003: 71-6.

47. Kaleem Z, Crawford E, Pathan MH et al. Flow Cytometric Analysis of Acute Leukemias Diagnostic Utility and Critical Analysis of Data. Arch Pathol Lab Med 2003; 97(5):319-22

48. Kresno SB, Haryanto SH, Kosasih AS et al. Immunophenotyping in leukemia and its diagnostic significance. Med J Indones 2004; 13: 195-202.

49. Orfao A, Ortuno F, Santiago M at al. Immunophenotyping of Acute Leukemias and Myelodysplastic Syndromes. Cytometry Part A-Wiley-Liss Inc 2004 ;58A: 62–71.

50. Qadir M, Barcos M, Stewart C et al. Routine Immunophenotyping in Acute Leukemia: Role in Lineage Assignment and Reassignment. Cytometry Part B (Clinical Cytometry) 2006; 70B:329–334.

51. Plesa G, Ciuperca V, Louvet L et al. Diagnostics of the AML with immunophenotypical data. Math. Model. Nat. Phenom 2008; 2(1): 104-123.

52. Mukda E, Pintaraks K, Komvilaisak P et al. Cytochemistry and Multi-color Flow Cytometric Immunophenotype for Diagnosis of Childhood Acute Leukemia. J Hematol Transfus Med 2011;21:23-31.

53. Roberts GT, Spence DG, Padmos MA, Sheth KV. Clink H, Ernst P.

Morphologic, immunophenotypic and cytogenetic patterns of adult acute myeloid leukemia in Saudi Arabia. Leukemia Research 1992;16:181-90.

54. Dalia A. Salem and Sherin M. Abd El-Aziz. Flowcytometric Immunophenotypic Profile of Acute Leukemia: Mansoura Experience. Indian Journal of Hematology and Transfusion 2012 June;28(2):89-96

55. Ihsan M. Osman, Amira A. K. Humeida, Osama Eltayeb, Elhadi et al.Flowcytometric Immunophenotypic Characterization of Acute Myeloid Leukemia (AML) in Sudan. International journal of hematological disorders 2015;2(1):10-17

56. Cesare Guglielmi, Maria Paola Martelli, Daniela Diverio et al.Immunophenotype of adult and childhood acute promyelocytic leukaemia: correlation with morphology, type of PML gene breakpoint and clinical outcome. A cooperative Italian study on 196 cases. British Journal of Haematology 1998;102:1035–1041.

57. Pei Lin,Suyang Hao,L. Jeffrey Medeiros et al. Expression of CD2 in Acute Promyelocytic Leukemia Correlates With Short Form of PML-RARα Transcripts and Poorer Prognosis.American Journal of Clinical Pathology 2004;121:402-407.

58. Mehdi Jahedi et al. Aberrant Phenotype in Iranian Patients with Acute Myeloid Leukemia. Adv Pharm Bull 2014 Mar; 4(1): 43–47.

59. B D Cheson, P A Cassileth, D R Head et al. R Brunning. Report of the National Cancer Institute-sponsored workshop on definitions of diagnosis and response in acute myeloid leukemia. JCO May 1990;8(5): 813-819.

60. Eddy Supriyadi, Pudjo H Widjajanto, Anjo JP Veerman et al. Immunophenotypic Patterns of Childhood Acute Leukemias in Indonesia Asian Pacific J Cancer Prev 2011 ;12(12):3381-3387.

61. Y Xu, R W McKenna, K S Wilson et al. Immunophenotypic identification of acute myeloid leukemia with monocytic differentiation. Asian Pacific Journal of Cancer Prevention. Leukemia 2006; 20:1321–1324.

62. Ashkan Emadi, Maria R . Baer. Acute Myeloid Leukemia in adults. Wintrobe's clinical hematology,13th edition, Lippincott Williams & Wilkins ;1593,94

63. Adriano Venditti, Giovanni Del Poeta, Roberto Stasi et al. Minimally differentiated acute myeloid leukaemia (AML-MO): cytochemical, immunophenotypic and cytogenetic analysis of 19 cases Sergio Amadori British Journal of Haematology 1994; Volume 88(Issue 4):784

64. Marie – Christine Bene, Michael Bernier, Rene Olivier Casasnovas et al.

Acute myeloid leukemia M0 : hematological, immunophenotyping and cytogenetic characteristics and their prognostic Significance: an analysis in 241 patients, British Journal of Hematology 26 thJan 2001;113:737-745.

65. Rajendra Kumar Nigam, Rajnikant Ahirwar, Reeni Malik et al. Clinicohematological profile of Acute Megakaryoblastic leukemia: Report of five cases. Journal of evolution of medical and dental sciences Oct,2013;Volume 2 (Issue 43):8349-8354.

66. Zahid Kaleem, Glenda White. Diagnostic criteria for minimally differentiated acute myeloid leukemia (AML-M0). Am J Clin Pathology 2001;115:876-884

67. Henry Y. Dong, Jia Xue Kung, Vatsala Bhardwaj et al. Flowcytometry rapidly identifies all acute promyelocytic leukemia with high specificity independent of underlying cytogenetic abnormalities. American journal of Clinical Pathology 2011;135:76-84.

68. Estella Matutes, Ricardo Morilla, Nahla Farahat et al. Definition of Acute Biphenotypic Leukemia, Haematologica 1997; 82:64-66.

69. Wolfgang Kern, Susanne Danhauser-Riedl, Richard Ratei et al. Detection of minimal residual disease in unselected patients with Acute Myeloid Leukemia using multiparameter flow cytometry to define leukemia – associated immunophenotypes and determine their Frequencies in normal bone marrow. Journal of hematology June 2003;vol 88(06):646-53

70. O Hrusak and A Porwit-MacDonald et al. Antigen expression patterns reflecting genotype of acute leukemias. Leukemia 2002;16:1233 -1258.

71. J T Reilly, B J Bain, J M England et al. The Role of cytology, cytochemistry, Immunophenotyping and cytogenetic analysis in the diagnosis of hematological neoplasm. Clin. Lab. Haem 1996;18:23.

72. Robert W McKenna. Multifaceted approach to the diagnosis and classification of acute leukemias. Clinical Chemistry August 2000;46(8):1252-1259.

73. Wintrobes Clinical Hematology, 12th edition.

74. D A Arber, R D Brunning, A Orazi. Acute myeloid leukemia and related precursor neoplasms. Who classification of tumours of Hematopoietic and lymphoid tissue. 4th edition;130

75. Frequent but nonrandom expression of lymphoid markers on de novo childhood acute myeloid leukemia.Experimental and Molecular Pathology Oct 2007;Volume 83(Issue 2):259–263.

76. Bakke AC. Morphologic and cytochemical characteristics of acute

promyelocytic leukemia.The Principles of Flow Cytometry. Laboratory medicine. 2001(april); 32(4)

77. Weinstein HJ. Acute Myeloid Leukemia. Childhood Leukemias 1999: 322-35

78. Miller KB, Daonst PR. Clinical Manifestation of Acute Myeloid Leukemia. In: Hoffman R, et al, editor. Haematology Basic Principles and Practice. 3rd edn. Pennsylvania: Churchill Livingstone; 2000.

79. WHO. Jaffe E, Harris N, Stein H, Vardiman J. Pathology and genetics of tumours of haematopoietic and lymphoid tissues. 2001.

ANNEXURES – 1

Myeloid	Myeloperoxidase by flow cytometry, immunohistochemistry, or cytochemistry
	or
Monocytic	differentiation—at least two of the following: nonspecific esterase,CD11c, CD14, CD64, lysozyme
T cell	Cytoplasmic CD3 or surface CD3
B cell	Multiple antigens are requiredStrong CD19 with at least one of the following strongly expressed: CD79a, cytoplasmic CD22, CD10
	or
	Weak CD19 with at least two of the following strongly expressed:CD79a, cytoplasmic CD22, CD10

ANNEXURE – 2

The WHO classification system for myeloid leukemias is summarized as follows:

AML with recurrent genetic abnormalities

- AML with t(8;21)(q22;q22); RUNX1-RUNX1T1
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11
- Acute promyelocytic leukemia with t(15;17)(q22;q12); PML-RARA
- AML with t(9 ;11)(p22;q23); MLLT3-MLL
- AML with t(6;9)(p23;q34); DEK-NUP214
- AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1
- AML (megakaryoblastic) with t(1;22)(p13;q13); RBM15-MKL1
- AML with mutated NPM1
- AML with mutated CEBPA

AML with myelodysplasia-related changes

Therapy-related myeloid neoplasms

AML not otherwise specified

- AML with minimal differentiation
- AML without maturation
- AML with maturation
- Acute myelomonocytic leukemia (AMML)
- Acute monoblastic/monocytic leukemia (AMoL)
- Acute Erythroid leukemias
    - Pure erythroid leukemia
    - Erythroleukemia, erythroid/myeloid
- Acute megakaryoblastic leukemia (AMkL)
- Acute basophilic leukemia
- Acute panmyelosis with Myelofibrosis

Myeloid sarcoma

Myeloid proliferations related to Down syndrome

- Transient abnormal myelopoiesis
- Myeloid leukemia associated with Down syndrome

Blastic plasmacytoid dendritic cell neoplasm

Annexure – 3

Immunophenotyping of AML with specific cytogenetic abnormalities (49)

t (8,21) MPO++, CD34 p+, CD13+, CD33+, CD19+, CD56+,CD65 +

t (15;17) MPO++, CD34 -/p+, CD13 het+, CD33++, CD117-/+, CD15-/dim+

inv (16) MPO++, CD34 p+, CD13 +++, CD33++, CD117 p+, CD65+

t (9;11) MPO dim+, HLA-DR+, CD34 -/+, CD13 dim+, CD33+, CD65-/+

p=partially, het=heterogeneously.

Annexure – 4

On the basis of molecular testing at the time of diagnosis, in non-APL AML, it is generally restricted to a limited number of scenarios:

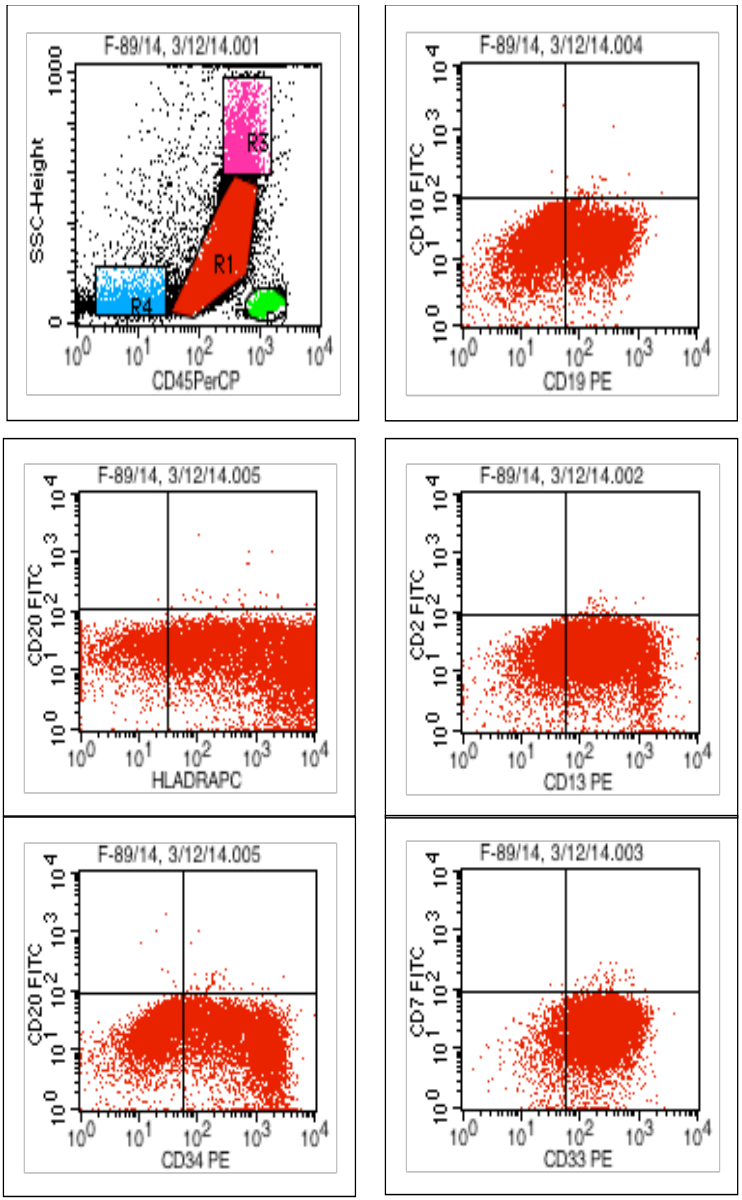
i. NPM1 and Flt3-ITD and Flt3-TKD testing in cytogenetically normal AMLs.

ii. c-Kit mutation testing in high Lkc or otherwise problematic t(8;21), inv(16), or t(16;16) cases

iii. Documented or presumed cases of CML in blast crisis

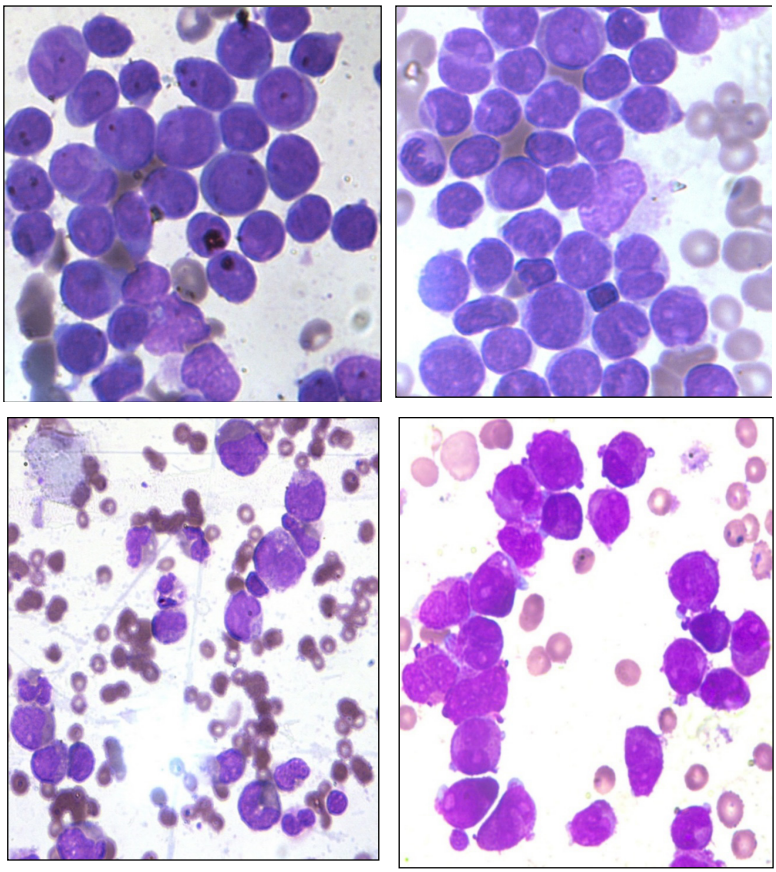
iv. Flt3 testing for clinical trial eligibility.

ANNEXURE 5


F-89/14, 3/12/14.001
SSC-Height
CD45PerCP
R1
R3
R4
F-89/14, 3/12/14.004
CD10 FITC
CD19 PE
F-89/14, 3/12/14.005
CD20 FITC
HLADRAPC
F-89/14, 3/12/14.002
CD2 FITC
CD13 PE
F-89/14, 3/12/14.005
CD20 FITC
CD34 PE
F-89/14, 3/12/14.003
CD7 FITC
CD33 PE

This flowcytometric analysis is of a case of AML M2.The blast population is gated with red color, lymphocytes by green color, granulocytes by pink and debris by blue color.

As we can infer from the analysis, there is expression of CD13, CD33, CD117, CD34, HLA-DR and MPO with aberrant expression of CD19. CD2, CD7, CD20 and CD10 are negative.



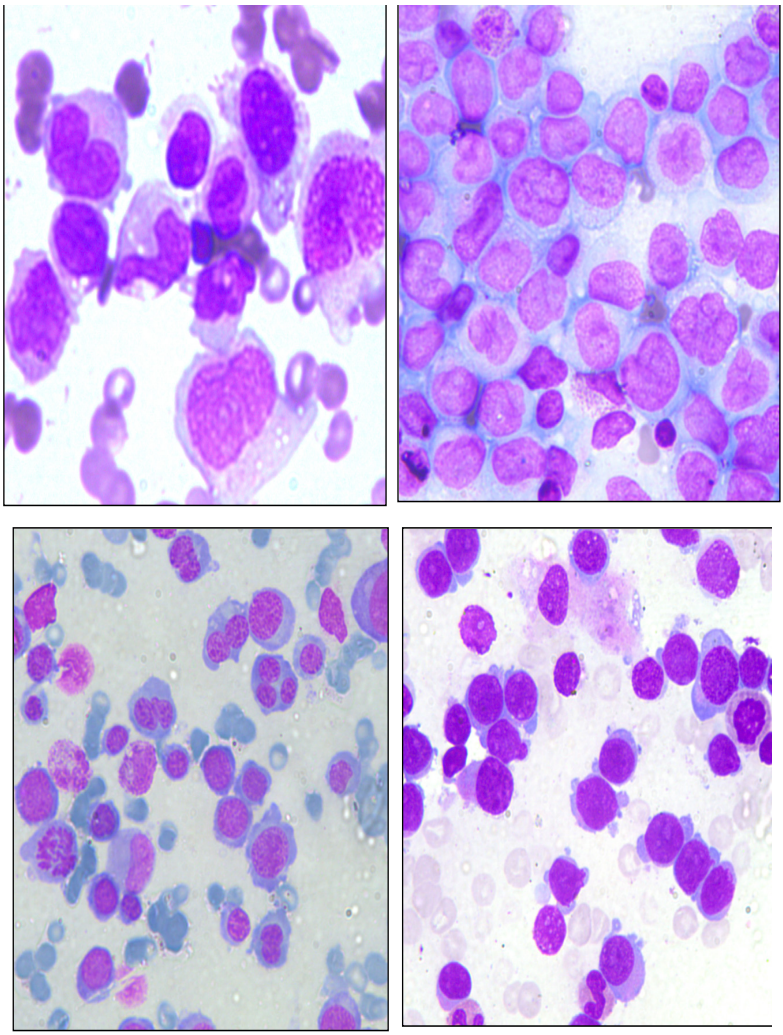
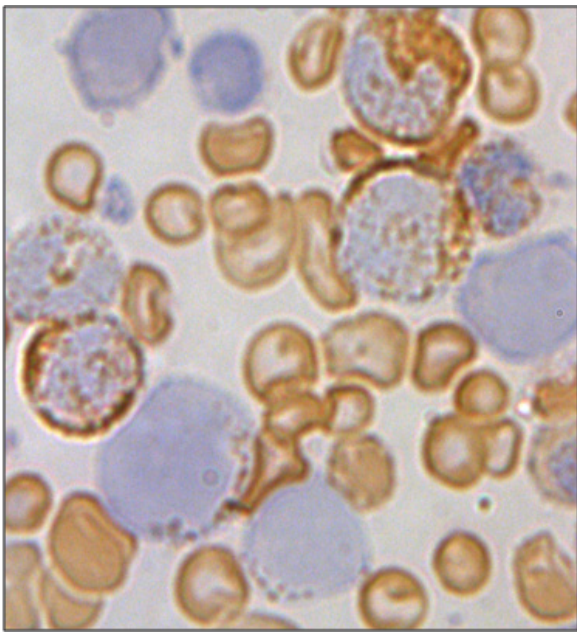


Fig1.M0 without evidence of myeloid maturation 2.M1>90%blasts <10% granulocytic maturation 3.M2 >10%granulocytic maturn4M3. lobulated Promyelocytes5&6.M4&M5 Monocytoid blasts wth lacy chromatin 7M6 Erythroid blasts with sieve chromatin8M7 Blasts with vacuolation&blebbing.



Myeloperoxidase Stain positive in Myeloblasts in AML.