# A Clinicopathological Correlation of Acute Leukaemias in relation to Immunophenotyping and Cytogenetics

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## Abstract

**Introduction:** Leukemia accounts for 0.15 – 0.6% of the total medical admissions in many general hospitals in India. Frequency of leukemia seen in India of Acute Myeloid leukaemia (AML) is 20 - 25% and Acute Lymphoblastic leukaemia (ALL) is 15-25%. The Annual incidence rate of AML and ALL are 5.6 and 30.9 per million population respectively.

**Aims:** To study the clinicopathological correlation in Acute myeloid and Acute Lymphoblastic leukaemias in relation to immunophenotyping and cytogenetics.

**Materials & Methods:** All newly diagnosed cases of acute myeloid leukaemia that presented to our hospital from January 2007 to July 2009 were included in this study. The peripheral blood and bone marrow were tested for surface membrane, cytoplasmic and nuclear antigens and were classified by the French-American- British (FAB) Cooperative Group Classification by using Romanowsky (Leishman and May Grunwald Giemsa[MGG]) stained smears and cytochemical stains.

**Results & Summary:** A series of available 100 cases of Acute Leukemia diagnosed during a period of 30 months (January 2007 to July 2009) were reviewed and various clinical, biochemical, immunophenotypic and cytogenetic parameters were assessed. 88 cases were subject to immunophenotyping and 60 cases were subject to cytogenetic analysis either by conventional Karyotyping, FISH (fluorescence *in situ* hybridization) and RT-PCR (Reverse transcriptase polymerase chain reaction). The antigen expressions by immunophenotype in acute myeloid and lymphoblastic leukemias were compared with age, Haemoglobin, Total WBC count, Platelet counts, Lactate dehydrogenase levels and abnormal karyotypes. Analytical statistics showed a significant correlation in the expressions of CD13, CD33, CD117 and CD64 in Acute Myeloid Leukemia and CD10, CD19, CD20 and CD22 in Acute Lymphoblastic leukemia and the expressions of CD13/CD117, CD3/CD10/CD22,CD3/CD5/CD2 and CD117/CD11c were related to the age, Haemoglobin, WBC count and Lactate dehydrogenase levels respectively ($p<0.05$).

**Conclusion**: We assessed the role of immunophenotyping and cytogenetics and their clinicopathological correlation with various haematological and biochemical parameters and found a statistically significant correlation with various parameters and supported expression of certain antigens and abnormal karyotypes correlate with a poor prognosis in Acute leukemias.

**Keywords:** Acute myeloid leukaemia, Acute Lymphoblastic leukemia, Acute Leukaemia, Immunophenotype, flow cytometry, cytogenetics.

## Introduction

The leukemias are a group of malignant disorders of the haemopoietic tissues characteristically associated with increased number of leucocytes in the blood or are a heterogeneous group of neoplasm arising from malignant transformation of haemopoietic cells.

Leukemic cells proliferate primarily in the bone marrow and lymphoid tissues where they interfere with normal haemopoesis and immunity. Ultimately they emigrate in to the peripheral blood and infiltrate other tissues. Thus leukemia's are progressive and fatal condition resulting in death most often from hemorrhage or infections. The course may vary from a few days or week to many years depending on the type.

Leukemia account for 0.15 – 0.6% of the total medical admission in many General hospitals in India.[9] Males are affected more frequently than females. Male to female ratio- in acute leukemia is 3: 2. Frequency of leukemia seen in India of AML is 20 – 25 % and ALL is 15 - 25%.[9] The Annual incidence rates of AML and ALL are 5.6 and 30.9 per million population respectively.[10]Acute lymphoblastic leukemia is primarily a disease of children and young adult, where as AML occurs primarily in adults. (ALL shows a peak incidence in the 1-5 age group).Each year, approximately 10,000 children (0-21 years) develop AML. Current epidemiological studies predict about 6.4-6.7 AML cases per million in the USA, while in China it is about 11 AML cases per million and in India 3.5 AML cases per million. The mechanisms underlying these differences have not yet been determined.[4]

In contrast to Children, Leukaemia accounts for a very small proportion of malignancy in adults. AML is much more common than ALL in Adults, with the incidence rising from 1 case/100,000 under the age of 50 to nearly10 cases/100,000 under the age of 80 years. In contrast the incidence of ALL is nearly 2cases/Million under the age of 50, rising to about 2 cases/100,000.ALL is four times more common than AML in children. An exception to this statistic is the neonatal period, when AML incidence shows a relative peak. The incidence of AML is stable until the teenage years, when a small increase in incidence is noted, followed by a steadily increasing incidence during adulthood. There is no difference in the incidence of AML in males when compared with females.[11]

In addition to ethnicity, environmental and genetic risk factors have been shown to influence the risk of developing AML and ALL. The most common genetic risk factor is Trisomy 21/Down's syndrome, which is associated with 15-fold increased risk of leukemia (both ALL and AML) during childhood. Environmental risk factors include Ionizing Radiation and exposure to Organic solvents, particularly Benzene. Other factors associated with increased

risk include parental occupational exposures to Hydrocarbons and Pesticides, maternal Alcohol use and Cigarette smoking during pregnancy.

The diagnosis and classification of leukemia rely on the simultaneous application of multiple techniques. Cytomorphology and histomorphology are combined with cytochemistry and multiparameter flow cytometry to assign the diagnostic sample to the correct entity. Furthermore, chromosomal analysis, often supplemented by fluorescence in situ hybridization (FISH), and molecular techniques, such as polymerase chain reaction (PCR), is needed to definitively confirm the diagnosis. A comprehensive and standardized algorithm for a diagnostic workflow and an effective and carefully designed combination of methods is essential to guarantee that all the required diagnostic information is gathered.[2]

This huge amount of laboratory assessment is necessary not only to diagnose and classify leukemia samples correctly but to detect biologically homogeneous entities that require specific treatment approaches. Thus, the detailed leukemia classification proposed by the French-American-British (FAB) Cooperative Group has been improved by thoroughly defined genetic and other characteristics, resulting in the new World Health Organization (WHO) classification. This also led to new prognostic markers and even to disease specific therapeutic approaches. The prime example for this strong link between a comprehensive diagnosis and a disease-specific treatment approach has been the use of all-*trans* retinoic acid (ATRA) in patients with acute promyelocytic leukemia.[2]

## Aims of the Study

1. To evaluate the immunophenotypic characteristics of acute leukemias.
2. To assess the cytogenetic features of acute leukemias.
3. To correlate clinical features with immunophenotyping and cytogenetics.

## Materials & Methods

### *Setting*

The study was conducted in Amrita Institute of Medical Sciences and Research Centre, Kochi, India, a tertiary care and teaching hospital**.**

### *Patients Selection*

Patients diagnosed at Amrita Institute of Medical Sciences as Acute leukemia according to WHO criteria were selected. The samples for biochemical and haematological investigations with bone marrow aspiration and trephine biopsy were received in the Department of Pathology during a period of 30 months (January 2007 to July 2009). A prospective and a retrospective study were done to assess the immunophenotypic characteristics and cytogenetics of acute leukemias along with various biochemical and hematological parameters and follow-up details were obtained from the medical records.

***Exclusion criteria***

1. Patients with Acute leukemia but without immunophenotyping and/or cytogenetic results.
2. CML transforming into acute myeloid leukemia.
3. MDS transforming into acute myeloid leukemia.

***Study Design***

A morphological evaluation was done from the Leishman and May Grunwald Giemsa stained peripheral smears and bone marrow aspirates using the French American British (FAB) classification of acute leukemias. A histopathological evaluation was done from the Haematoxylin and Eosin stained paraffin sections of bone marrow trephine biopsies.

Special relevant cytochemical stains were performed on the bone marrow aspirates in all cases. In each case along with pathology reports, electronic medical records were reviewed for pertinent clinical information including age, gender, initial clinical presentation, number of bone marrow blasts, immunophenotype, cytogenetics, Haemoglobin level, Total White blood cell count, platelet count, Lactate Dehydrogenase levels, administration of chemotherapy, remission or relapse of the disease and status at the most recent follow-up.

During the defined period of the study, 183 acute leukemias patients had been evaluated and were started on appropriate chemotherapy. Of these, only 100 patients had their immunophenotyping and/or cytogenetic results. 33 patients were lost to follow up after investigations, 10 died with disease without completing the full course of chemotherapy and 83 patients had no immunophenotypic and cytogenetic analysis and hence these 83 cases were excluded from the study. Finally, peripheral smear, bone marrow aspiration smears and slides of bone marrow trephine biopsy of 100 cases were studied. Clinical, biochemical and pathological correlation were done and cases satisfying FAB and WHO criteria for acute leukemia were included. All the cases were looked for number (% of total nucleated cells) of blasts in peripheral blood as well as bone marrow aspiration, and distribution and pattern of leukemic blasts in bone marrow biopsy.

Bone marrow aspiration smears were prepared by 'push' method & were stained by Giemsa stain. The 'push' method is called so because the spreader slide is pulled into the drop of blood and the smear is made by pushing the blood along the slide. Special cytochemical stains like Myeloperoxidase(MPO), Periodic acid Schiff (PAS) and Non-Specific esterase where done where deemed necessary. Bone marrow biopsies were decalcified in a solution of formol acid as well as formal acid alcohol. Sections were stained with hematoxylin and eosin (H&E), periodic acid Schiff, and Gomori's stain for reticulin fibres.

All samples for immunophenotyping were collected in a vacutainer (BD Vacutainer) containing Heparin and sent to Ranbaxy Laboratories, Mumbai or OncQuest, New Delhi for analysis.

**Analytical Statistics**

All the data were analyzed using the SPSS 11.0 version and relevant tests of significance applied wherever necessary. The Chi- square test, Mann Whitney test and Wilcoxon's rank

sum test were applied. Multivariate analysis was not performed because of the smaller number of samples in the study.

## Results

The present study was done by reviewing medical records and peripheral smear, Bone marrow aspirates and bone marrow biopsy slides of 100 patients with Acute Leukemia (Figures 1-5). Initially clinical, biochemical and morphologic features and later the prognostic implications of various factors were assessed. Follow-up details of 67 patients were obtained. The results are summarised in Tables 1-5.

### *Age and Sex*

The age of patients at the time of diagnosis ranged from 1 year to 78 years. The maximum number of patients belonged to 1$^{st}$ & 2$^{nd}$ and 3$^{rd}$ & 4$^{th}$ decade of age group (37 & 35 cases respectively). The average age of the patients was 29.58 years. The majority were males constituting 58 % and the male to female ratio was 1.38: 1.

### *Correlation of immunophenotype and Clinical features*

The lowest haemoglobin level was noted in AML-M1 with a value of 3.72g/dl while the highest level was seen in AML-M2 with a value of 12.7g/dl (Table 5). The lowest WBC count was seen in AML-M7 with a value of 0.6k/UL and the highest count was seen in AML-M3 with a value of 272k/UL (Table 5).

The lowest platelet count was seen in AML-M3 with a value of 15k/UL while the highest platelet count was seen in AML-M1 with a value of 484 k/UL (Table 5).

The highest Lactate dehydrogenase levels were noted in AML-M7 with a mean of 3140 IU/l and the lowest level was seen in AML-M1 with a level of 110k/UL (Table 5).

The lowest and highest haemoglobin level was noted in ALL-L2 with a value of 3.95g/dl and 14.04g/dl respectively (Table 5).

The lowest and highest WBC count was seen in ALL-L2 with a value of 1.21k/UL and 278k/UL respectively (Table 5).

The lowest platelet count was seen in ALL-L2 at 6.85k/UL and the highest count was seen in ALL-L1 at 311k/UL (Table 5).

The lowest Lactate dehydrogenase levels were seen in ALL-L2 at 111IU/l and the highest level was seen in ALL-L1 at 22035IU/l (Table 5).

Our results (Table 12) showed that the expressions of CD13/CD117, CD3/CD10/CD22,CD3/CD5/CD2 and CD117/CD11c were related to the age, Haemoglobin, WBC count and Lactate dehydrogenase levels respectively ($p<0.05$). None of the antigens had any significant correlation with the platelet counts in the peripheral blood.

### *Correlation between immunophenotype and Cytogenetics*

No statistical correlation could be obtained between the Immunophenotype and the abnormal karyotypes. The reason could be due to the smaller number of patients who had an abnormal karyotype and who also had immunophenotyping (Table 12).

### *Bone marrow blasts*

The least number of bone marrow blasts in our AML cases was seen in AML-M7 at 20% while the highest number of promyelocytes was seen in AML-M3 at 98%. The least number of bone marrow blasts in our ALL cases was seen in ALL-L2 at 18% and the highest number of blasts was seen in ALL-L3 at 97%.

## Discussion

Immunophenotypic studies have an established place in the diagnosis and classification of acute leukaemia.[12] The availability of murine monoclonal antibodies that are reactive with lymphoid and myeloid associated surface epitopes, coupled with relatively user friendly and inexpensive flow cytometers, has made the use of immunophenotypic analyses in acute leukaemia easily accessible to many laboratories.

Numerous reports on the phenotypic characteristics of acute leukaemia have been generated. Most of the studies have expanded our understanding of these diseases, and have facilitated the recognition of certain types of acute leukaemia, such as minimally differentiated AML and B and T cell ALL. Applying extensive panels of antibodies to large numbers of acute leukemias, however, also has dramatically increased the heterogeneity of acute leukaemia. At times this has created confusion and uncertainty about the significance of the relationship between phenotype and biologic behaviour, and has complicated classification in a small proportion of cases.[3]

Our study generates immunophenotypic profiles that may help to better define the range of antibody reactivity for many types of acute leukaemia. The data were collected from samples referred for characterization and diagnosis of acute leukaemia. There have not been many studies done in India which have analysed the Immunophenotypic and Cytogenetic features of Acute leukaemias i.e. both Myeloid and Lymphoblastic; hence the importance of this study.

### *Acute myeloid Leukaemia (AML)*

In this study, we analysed the immunophenotype of 38 cases of Acute Myeloid leukaemia. Consistent with the previous studies in literature[1,5,7,13-16], our study showed that CD33, CD13, CD117 and CD64 were the most commonly expressed myeloid antigens.

Early myeloblasts express CD34 and HLA-DR but these are lost by the promyelocyte stage.[24] 52.63% of our cases were positive for CD34 and the highest positivity was seen in the AML-M1 and AML-M2 subtypes.

1 out of 4 cases of AML-M5 in our study expressed CD14 and none of the AML-M4 cases showed CD14 expression. This antigen expression is not restricted to acute leukaemia with monocytic differentiation.

Acute Promyelocytic leukaemia (AML-M3) is an unusual and distinctive disease clinically, morphologically, immunophenotypically and even genotypically.[21] As previously reported, the majority of cases of AML express HLA-DR.[8,13,15] The clear exception to this rule is AML-M3,in which only rare cases are reported to be positive.[13,22]

In this study, 2 out of 4 cases of AML-M3 expressed HLA-DR whereas 1 case showed CD34 expression. None of our AML-M3 cases showed CD2 expression, though positivity for this T cell marker has been well documented in AML-M3, and the presence of this antigen has even been associated with specific breakpoints within the t(15;17) translocation.[23] In our study, 4 cases of AML-M3 showed the characteristic translocation t(15,17) detected by conventional Giemsa banding techniques or PML RARA protein by Reverse Transcriptase PCR or FISH.

***Comparisons with other studies***

Hanson et al [13], Terstappen LWM et al [49], Roberts et al [15], San Miguel et al [16], Zheng et al [1], Kaleem et al [17] and Auewarakul et al [50] demonstrated that CD33, CD13, CD117 and CD64 were the most commonly expressed myeloid antigens in their studies of acute myeloid leukaemia.

The immunophenotypic signature of these acute leukemias is expression of one or more myeloid antigens, which was seen in nearly all the cases. A lack of myeloid antigen positivity does not absolutely exclude AML, though our study showed no such findings.

Traweek [6] in his study of 207 cases of AML found 3 cases that failed to mark as myeloid despite the presence of Myeloperoxidase activity and morphologically was consistent with AML-M2.Although unusual, myeloid antigen negativity in *bona fide* AML has been reported previously.[13,16-17] In general, little-mixed lineage antigen expression was seen in our series of AML cases. When present, these small expressions of lineage promiscuity should not be construed as sufficient evidence to warrant a diagnosis of mixed-lineage leukaemia.[18]

Ghosh et al [26] in their study of 260 cases of AML at Tata memorial Hospital, Mumbai observed a similar finding, although their study showed a positivity of 25% for CD34.

Borowitz et al[27] have reported a positivity of CD34 (45%) in the more immature leukemias and a strong association with loss or partial deletion of chromosome 7 and 5. The blasts of AML-M2 without the t(8;21) may also express CD34, CD65, HLA-DR. However the intensity of CD33 and CD13 antigen expression by these blasts usually exceeds that of t(8;21) positive blasts. CD19 is rarely detectable whereas T- cell associated antigen CD2 and 7 are more often noted on the blasts of AML-M2 without t(8;21).[25]

Initially thought to be a T cell antigen, CD7 is now known to be present in AML.[19] CD7 expression appears to be associated with blast cell immaturity,[19] because the percentage of positive cases was greatest in AML-M1, AML-M5 and AML-M7.CD3 and CD4 were the

other antigens detected. Traweek[6] found greatest expression of CD7 in AML-M0, AML-M1 and AML-M5a with CD2 being the other antigen detected. Zheng et al [1] studied 180 cases of AML and showed that CD7 was the most commonly expressed followed by CD2, CD19 and CD22.B-cell antigen expression was almost exclusively CD19, and was predominantly seen in AML-M1. Kita and colleagues showed an association between CD19 expression and AML-M2, and also has been linked with a specific cytogenetic abnormality, the t(8;21) translocation.[20] CD19 has been associated previously with AML-M5,but none were detected in our study.

Traweek [6] in his study of 207 AML cases found expression of CD14 in 6% of AML-M2 cases and 1 case of AML-M3.Upto 25% of M1 and M2 AML's have been previously reported to express this antigen,[15-16] but CD14 expression in AML-M3 is apparently rare.[6]

Other studies clearly indicate that expression of HLA-DR may occur in other subtypes of AML.[24]

There is little data on cytogenetic features of c-*kit* positive AML. In Bradstock et al [28] series of 233 adult AML patients, chromosomal aberrations were more frequent in CD117 positive AML than in CD117 negative AML (41% vs. 60%, $p < 0.05$). In addition, they found CD117 expression in 10/10 AML cases with the t(8;21) aberration.

The Glycophorin A (GLY-A) antigen is usually detected in the more differentiated forms of Pure Erythroid Leukemia. In our study, 1 case each of AML-M1 and AML-M2 showed positivity for Glycophorin A while this antigen was not seen in our AML-M6 case. Zheng et al [1] in their study found expression of this antigen in 1 case each of AML-M2 and AML-M4 respectively.

***Karyotyping in AML***

Consistent with previous studies, 4 of our cases of AML-M3 showed the characteristic translocation t(15,17) detected by conventional Giemsa banding techniques or PML-RARA protein by Reverse Transcriptase PCR or FISH. 4 cases showed a normal karyotype but showed PML-RARA fusion protein by RT-PCR/FISH and 1 case showed a Monosomy 10&21.

There was a case of AML-M2 showing the characteristic translocation t(8;21),1 case with a Monosomy 8&11 and 3 cases with normal karyotype. Of the 9 AML-M1 cases, 5 showed a normal karyotype, 1 had a t(9;22) translocation,1 had Trisomy 21 and 2 patients had a complex karyotype.

All our cases of AML-M4 and AML-M5 showed a normal karyotype, though the numbers of cases are very small to make any significant conclusion. None of our AML-M6 and AML-M7 cases showed any abnormal karyotypes.

Zheng et al [1] in their study of 180 AML patients had Karyotyping results of 60 AML-M2 cases, of which 22 had a normal karyotype, 16 patients with a t(8;21) translocation and 22 with other karyotypes.3 cases showed complex karyotypes and there was a 3 patients showing a 11q23,Trisomy 8 and a chromosome 5/7 abnormality respectively. Zheng et al [1] studied 17 cases of AML-M1 and found 7 with a normal karyotype,1 with chromosome 5/7 abnormality and 6 with complex karyotypes .Tong et al [29] in their study of 192 Chinese

patients with AML,9 cases of AML-M1 of which 7 showed a normal karyotype and 2 with other karyotypes. Zheng et al [1] and H Tong et al [29] have noted 11q23 abnormalities and inv(16) in their AML-M4 and AML-M5 cases.

Currently chromosomal analysis forms an important component of the diagnostic, prognostic (favourable, intermediate and unfavourable risk groups) and biologic studies of AML. Chromosomal aberrations are seen in 90% of these patients.[48] The recent WHO classification has also stressed on the importance of cytogenetic abnormalities and multilineage dysplasia in the subtyping of leukemias. However facilities for chromosomal studies are not readily available in our country. In its absence, the FAB classification based on morphology, cytochemistry, Immunophenotyping remains extremely useful in arriving at a correct diagnosis.

Zheng et al [1] in their study showed that the expressions of CD4/Tdt,CD7/CD14 and CD4/CD14/CD56 were related to age, WBC counts, and Platelet counts in peripheral blood, respectively ($p<0.05$),while the expression of these antigens poorly associated with level of haemoglobin. Zheng et al [1] in their study of 207 AML's showed that the expressions of CD22, CD56 and Tdt seemed to be highly related to the abnormal chromosomal karyotype ($p<0.05$). Particularly abnormal karyotypes were found in all of their 10 cases of patients of CD22.

Ghosh et al [26] in their study of 207 AML patients observed that the lowest WBC counts was seen in AML-M3 and the highest WBC counts was seen in AML-M1 followed by AML-M4 and AML-M5. Ghosh et al[26] in their study noted that the least number of bone marrow blasts in AML-M4 and the highest number was in AML-M1.

### *Acute Lymphoblastic leukemia*

The separation of ALL into B- and T-cell types is clinically important, with both therapeutic and prognostic implications.[30,31] The cases of ALL in this study were therefore divided into three subtypes, one based on the FAB classification into ALL-L1,ALL-L2 and ALL-L3 and the other based primarily on immunophenotypic findings.

The precursor B-cell ALL's all expressed CD19, by definition. In addition, all cases were HLA-DR positive. Otherwise, these leukemias were the most heterogeneous leukemias in this study. The majority of cases expressed CD10, which is a marker of longstanding and well-known prognostic value.[32] On the other hand; CD10 negativity has been associated with a poor prognosis.[33] Our study showed one such case, which was CD10 negative and achieved clinical remission by chemotherapy. As has been previously reported, CD20 was more common in ALL-L2 than in ALL-L1 cases.[6,34]

In Contrast to AML, a significant majority of Precursor B-ALL cases in our study failed to express the common leukocyte antigen CD45.This is in stark contrast to the studies done by Traweek[6] and Caldwell et al [35], who showed that in contrast to AML, only a significant minority failed to express the CD45 antigen, a finding of possible prognostic significance. Myeloid antigens were commonly present, however, and were seen singly or in pairs in 19% of cases. No biphenotypic leukemias were identified by EGIL criteria. Traweek[6] found a similar expression in 23% of Precursor B –cell ALL cases. As in AML, the presence of these aberrantly expressed antigens should not preclude an otherwise straightforward diagnosis of

Precursor B-cell leukemia. The expression of T cell antigens is infrequently detected in this type of leukemia.[31,34]

B-cell ALL is a rare disease, and is the least common subtype of ALL. Classical FAB-L3 morphology is associated with a B cell phenotype. Morphologic features that are characteristic of non-L3 blasts however, also can be seen in this subtype of acute leukemia.[36,38] 8 cases in this study expressed B-cell ALL phenotype, but did not display an ALL-L3 appearance. Cytogenetic studies were performed on two of the cases which revealed a t(9;22) and t(4;11) cytogenetic abnormality. Immunophenotypic studies are few, but most cases previously reported and all cases of B-ALL in this study expressed CD19 and HLA-DR and were CD34 negative.2 of the 10 cases expressed TdT positivity. B-ALL with t (9; 22) is typically CD10+,CD19+ and TdT+. None of the ALL-L3 cases showed t(8;14) translocation and SIg was not routinely done in the flow cytometry panel.

In both children and adults, t(9;22) ALL has the worst prognosis among patients with ALL. ALL with MLL translocations and most especially t(4;11) ALL typically have a CD19+,CD10 negative,CD24 negative pro-B immunophenotype, also positive for CD15.[39-40] Our case with this translocation showed cells expressing CD19 and CD10. 2 cases expressed CD15 and one case expressed CD5, an unusual finding, but none were positive for other T cell or myeloid markers.CD10 was detected in majority of the cases, which is typical.[36-37]

The phenotypic heterogeneity of T-cell neoplasms is well documented and covers the range of phenotypes expressed during thymic differentiation.[41-43] Most cases will express more than one T-lineage marker. Aberrant deletion of one or more pan T-cell antigens is common in this disease, however, and maybe a helpful diagnostic finding.[44] All the 3 cases of T-ALL showed deletion of one or more of the 4 Pan T-cell antigens used (CD2,CD3, CD5 & CD7). CD5 was the pan-T-cell antigen most often expressed by the T-cell cases in our study. CD4/CD8 is not included in the conventional ALL panel that we sent for immunophenotyping ,hence whether these cases were double positive or double negative was not known. Traweek[6], Kaleem et al[5] and Vodinelich et al [47] in their series showed that CD7 was the most often expressed pan-T-cell antigen. CD7 is also the antigen that is most commonly deleted in these acute leukemias.[46] No CD7 positivity was detected in any Pre-B- or B-cell ALL, which is typical.[45] The HLA-DR antigen is not expressed in T-cell ALL,[42] which is another phenotypic feature that distinguishes this acute leukemia from Pre-B-cell ALL and B-cell ALL. None of the cases of T-cell ALL in this study were HLA-DR positive thus concurring with other studies in literature. CD10 has been reported in 20-30% of T-cell ALL's, [41] a frequency approximately similar to that seen in this series. No myeloid antigen expression was seen in T-Cell ALL of our series; though this has been reported in literature.[46] Expression of myeloid antigens may be a potential source of confusion considering the occasional presence of CD2 and CD7 in AML. One case showed aberrant expression of CD19 and this patient achieved CR with standard chemotherapy regimens.

### *Karyotyping in ALL*

70% of our ALL cases showed a normal karyotype. There was one case each showing a t(4;11) and t(1;7) chromosomal abnormality. The patient with the t(1;7) chromosomal abnormality had a relapse of the disease in spite of being given chemotherapy and then expired. The patient with the t(4;11) chromosomal abnormality is currently under follow up

without any relapse of the disease. Three patients had a Philadelphia chromosome positive ALL, of which 2 had a relapse of the disease and one achieved clinical remission (CR).

B-ALL with t(9;22) chromosomal abnormality have a poor prognosis.4 patients had a complex karyotype, of which 2 had a relapse and 1 expired. One patient achieved CR and one was lost to follow up. These leukemias generally carry a poor prognosis. Overall the numbers of cases with cytogenetics were few to arrive at any meaningful conclusion.

## Conclusion

We assessed the role of immunophenotyping and cytogenetics and their clinicopathological correlation with various haematological and biochemical parameters and found a statistically significant correlation with various parameters and supported that expression of certain antigens and abnormal karyotypes correlate with a poor prognosis in Acute leukemias.

We recommend the following:

 Immunophenotyping and cytogenetics should be performed routinely in all cases of Acute myeloid and Lymphoblastic leukemias.

 A correlation must also be done with various biochemical and haematological parameters.

 Furthermore, a standard scoring system has to evolve considering various parameters to predict the aggressive nature of acute leukemia at the initial evaluation of first aspirate and biopsy.

 Need for further studies in Acute leukaemias should be done.

## Limitations of study

1. Not all 100 patients were subject to Immunophenotypic and cytogenetic analysis because of the financial constraints involved with test costs.

2. Survival analysis of these patients could not be obtained as many were lost to follow up during the study period.

3. Overall, the numbers of cases with cytogenetic analysis were few to arrive at any meaningful conclusion

**Conflict of Interest:** None declared.

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**Table 1:** Positive rate and p value in 38 cases of Acute Myeloid leukemia according to immunophenotype

| Antigen | Positive rate | p value |
|---|---|---|
| **CD34** | 52.63% | 0.979 |
| **HLA-DR** | 81.58% | 0.439 |
| **CD13** | 86.84% | <0.001 |
| **CD33** | 92.10% | <0.001 |
| **CD117** | 57.89% | <0.001 |
| **MPO** | 7.89% | 0.082 |
| **CD15** | 10.53% | 0.398 |
| **CD64** | 15.79% | 0.005 |
| **CD11c** | 10.52% | 0.034 |
| **CD38** | 2.63% | 0.441 |
| **CD42a** | 2.63% | 0.441 |
| **CD45** | 57.89% | 0.039 |
| **Gly-A** | 5.26% | 0.581 |

Statistical test used: Chi-square test

The p values of CD13, CD33, CD117 and CD64 were statistically significant suggesting that they are useful markers in the immunophenotypic diagnosis of AML.(p value <0.05)

The main antigens expressed in acute myeloid leukemia included the following:
a) Myeloid antigens: CD13, CD33 and CD64 were expressed in the vast majority of AML cases at 86.84%, 92.1% and 15.79% respectively.
b) MPO was negative in all AML M0, M4, M5, M6 and M7 cases.
c) Stem/progenitor cell markers: HLA-DR, CD117 and CD34 were expressed at the overall rate of 81.58%, 52.63% and 57.89% respectively.
d) 1/4 cases of AML-M3 expressed CD34 and 2/4 cases of AML M3 expressed HLA-DR.
e) Aberrant Lymphoid markers: CD7 was the most commonly expressed lymphoid marker (10.5%) followed by CD4 (7.89%) and CD19 & CD3 (2.63%).
f) Other markers: CD15, CD11c, CD38, CD42a, CD45 and Gly A were expressed at the rate of 10.53%, 10.52%, 2.63%, 2.63%, 57.89% and 5.26% respectively.
AML- Acute myeloid leukaemia
MPO- Myeloperoxidase
HLA-DR- Human leucocyte antigen-D related
Gly-A- Glycophorin A

**Table 2:** Positive rate and p value in 49 cases of Acute Lymphoblastic leukemia by immunophenotype

| Antigen | Positive rate | p value |
|---|---|---|
| **CD10** | 89.8% | <0.001 |
| **CD19** | 93.88% | <0.001 |
| **CD20** | 18.36% | 0.004 |
| **CD22** | 79.6% | <0.001 |
| **CD34** | 51.02% | 0.979 |
| **HLA-DR** | 87.76% | 0.439 |
| **CD3** | 6.12% | 0.626 |
| **CD5** | 10.2% | 0.063 |
| **CD7** | 4.08% | 0.232 |
| **CD45** | 32.65% | 0.039 |
| **TdT** | 10.2% | 0.063 |
| **CD13** | 6.12% | - |
| **CD33** | 20.4% | - |

Statistical test used: Chi-square test

The p values of CD 10, CD19, CD20, CD 22 and CD45 are statistically significant (p value <0.05) suggesting that they are useful immunophenotypic markers in the diagnosis of ALL.

The main antigens expressed in Acute Lymphoblastic leukemia were as follows:
a) Lymphoid antigens: CD10, CD19, CD20 and CD22 were expressed in a number of cases at 89.8%, 93.88%, 18.36% and 79.6% respectively.
b) The T cell markers CD3, CD5 and CD7 were expressed at the rate of 6.12%, 10.2% and 4.08% respectively.
c) Stem cell/progenitor cell markers: HLA-DR and CD34 were expressed at the overall rate of 87.76% and 51.02% respectively.
d) CD45 and TdT were expressed at the rate of 32.65% and 10.2% respectively.
e) Aberrant Myeloid antigens: CD33 was the most commonly expressed myeloid marker (20.4%) followed by CD13 at 6.12%.
ALL- Acute Lymphoblastic leukaemia
HLA-DR- Human leucocyte antigen-D related
TdT- Terminal Deoxynucleotidyl Transferase

**Table 3:** Karyotyping in 30 cases of acute myeloid leukaemia by FAB subtype

| Karyotype | AML -M0 | AML -M1 | AML -M2 | AML -M3 | AML -M4 | AML -M5 | AML -M6 | AML -M7 |
|---|---|---|---|---|---|---|---|---|
| **Normal karyotype** | 2 | 5 | 3 | 4 | 1 | 2 | 1 | 1 |
| **Monosomy 10&21** | | | | 1 | | | | |
| **Monosomy 8&11** | | | 1 | | | | | |
| **t(15;17)** | | | | 4 | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **t(8;21)** | | | 1 | | | | | |
| **t(9;22)** | | 1 | | | | | | |
| **Trisomy 21** | | 1 | | | | | | |
| **Complex karyotype** | | 2 | | | | | | |

**Note**: The 4 cases of AML-M3 which showed a normal karyotype by conventional methods, showed the presence of PML-RARA fusion protein by FISH/RT-PCR.

FAB-French-American-British
FISH-fluorescence *in situ* hybridization
RT-PCR- Reverse transcriptase polymerase chain reaction
PML-RARA- Promyelocytic leukemia-retinoic acid receptor α

**Table 4:** Karyotyping in 30 cases of Acute Lymphoblastic leukaemias by FAB subtype

| Karyotype | ALL-L1 | ALL-L2 | ALL-L3 |
|---|---|---|---|
| **Normal karyotype** | 3 | 17 | 1 |
| **t(4;11)** | | 1 | |
| **t(1;7)** | | 1 | |
| **t(9;22)** | 2 | 1 | |
| **Complex karyotype** | 1 | 2 | 1 |

ALL- Acute Lymphoblastic leukaemia
FAB-French-American-British

**Table 5:** Haematological and biochemical parameters of 48 Acute Myeloid leukaemia cases and Haematologic parameters of 52 cases of Acute Lymphoblastic Leukaemia

| | LDH(IU) | | Hb(g/dl) | | WBC($10^9$/l) | | Platelets($10^9$/l) | |
|---|---|---|---|---|---|---|---|---|
| **Subtype** | **Mean** | **Range** | **Mean** | **Range** | **Mean** | **Range** | **Mean** | **Range** |
| **AML-M0** | 8.94 | 7.57-10.3 | 1.68 | 1.08-2.27 | 258.5 | 227-290 | 122 | - |
| **AML-M1** | 8.21 | 3.72-10.9 | 26.93 | 1.02-172 | 106.02 | 15.8-484 | 495.5 | 110-1063 |
| **AML-M2** | 9.92 | 6.99-12.7 | 21.68 | 1.92-42.8 | 91.9 | 17.8-293 | 727.67 | 223-1908 |
| **AML-M3** | 8.17 | 4.47-10.3 | 36.06 | 2.22-272 | 78.22 | 15-211 | 575.33 | 206-1383 |
| **AML-M4** | 9.85 | 9.30-10.4 | 21.64 | 6.57-36.7 | 44.15 | 33.1-55.2 | 682.5 | 463-929 |
| **AML-M5** | 9.32 | 8.14-10.6 | 79.32 | 7.47-219 | 101.6 | 46.8-153 | 581.75 | 404-721 |
| **AML-M6** | 9.05 | - | 6.36 | - | 64 | - | 847 | - |
| **AML-M7** | 7.40 | 6.76-8.03 | 7.25 | 0.6-13.9 | 154.5 | 137-172 | 1805 | 470-3140 |

| | Hb(g/dl) | | WBC($10^9$/l) | | Platelets($10^9$/l) | | LDH(I.U.) | |
|---|---|---|---|---|---|---|---|---|
| **Subtype** | **Mean** | **Range** | **Mean** | **Range** | **Mean** | **Range** | **Mean** | **Range** |
| **ALL-L1** | 8.49 | 5.18-11.8 | 26.44 | 1.79-99.4 | 161.5 | 14.5-311 | 3565.6 | 375-22035 |
| **ALL-L2** | 8.07 | 3.95-14.04 | 53.84 | 1.21-278 | 85.99 | 6.85-229 | 3823.5 | 111-5025 |
| **ALL-L3** | 7.14 | 5.65-8.16 | 8.76 | 1.52-16 | 32.85 | 20-45.7 | 588.5 | 423-754 |

AML- Acute myeloid leukaemia
ALL- Acute Lymphoblastic leukaemia
LDH-Lactate dehydrogenase

**Table 6:** Immunophenotyping in 38 patients of Acute Myeloid Leukemia according to FAB Subtype

| Antigen | M0(cases) | M1(cases) | M2(cases) | M3(cases) |
|---|---|---|---|---|
| **CD34** | 2/2 | 11/17 | 3/6 | 1/4 |
| **HLA-DR** | 1/2 | 15/17 | 5/6 | 2/4 |
| **CD13** | 2/2 | 13/17 | 6/6 | 4/4 |
| **CD33** | 2/2 | 15/17 | 6/6 | 4/4 |
| **CD117** | 1/2 | 10/17 | 3/6 | 3/4 |
| **MPO** | 0/2 | 2/17 | 1/6 | 0/4 |
| **CD15** | 0/2 | 2/17 | 1/6 | 0/4 |
| **CD64** | 0/2 | 3/17 | 1/6 | 0/4 |
| **CD11c** | 0/2 | 3/17 | 1/6 | 0/4 |
| **CD38** | 0/2 | 1/17 | 0/6 | 0/4 |
| **CD42a** | 0/2 | 0/17 | 0/6 | 0/4 |
| **CD45** | 2/2 | 9/17 | 4/6 | 3/4 |
| **Gly-A** | 0/2 | 1/17 | 1/6 | 0/4 |
| **CD61** | 0/2 | 0/17 | 0/6 | 0/4 |

All values of positive expression were expressed as positive/studied cases.

| Antigen | M4(cases) | M5(cases) | M6(cases) | M7(cases) |
|---|---|---|---|---|
| **CD34** | 1/2 | 1/4 | 0/1 | 1/2 |
| **HLA-DR** | 2/2 | 3/4 | 1/1 | 1/2 |
| **CD13** | 2/2 | 4/4 | 1/1 | 1/2 |
| **CD33** | 2/2 | 1/4 | 1/1 | 1/2 |
| **CD117** | 2/2 | 4/4 | 1/1 | 1/2 |
| **MPO** | 0/2 | 0/4 | 0/1 | 0/2 |
| **CD15** | 0/2 | 1/4 | 0/1 | 0/2 |
| **CD64** | 0/2 | 2/4 | 0/1 | 0/2 |

| | | | | |
|---|---|---|---|---|
| **CD11c** | 0/2 | 0/4 | 0/1 | 0/2 |
| **CD38** | 0/2 | 0/4 | 0/1 | 0/2 |
| **CD42a** | 0/2 | 0/4 | 0/1 | 1/2 |
| **CD45** | 0/2 | 2/4 | 1/1 | 1/2 |
| **Gly-A** | 0/2 | 0/4 | 0/1 | 0/2 |
| **CD61** | 0/2 | 0/4 | 0/1 | 1/2 |

All values of positive expression were expressed as positive/studied cases.

**Table 7:** Abberant antigen expression in 38 patients of Acute Myeloid Leukemia by FAB Subtype

| Antigen | M0(cases) | M1(cases) | M2(cases) | M3(cases) |
|---|---|---|---|---|
| **CD3** | 0/2 | 1/17 | 0/6 | 0/4 |
| **CD7** | 0/2 | 3/17 | 0/6 | 0/4 |
| **CD19** | 0/2 | 1/17 | 0/6 | 0/4 |
| **CD4** | 0/2 | 0/17 | 1/6 | 0/4 |

All values of positive expression were expressed as positive/studied cases.

| Antigen | M4(cases) | M5(cases) | M6(cases) | M7(cases) |
|---|---|---|---|---|
| **CD3** | 0/2 | 0/4 | 0/1 | 0/2 |
| **CD7** | 0/2 | 1/4 | 0/1 | 1/2 |
| **CD19** | 0/2 | 0/4 | 0/1 | 0/2 |
| **CD4** | 0/2 | 2/4 | 0/1 | 0/2 |

All values of positive expression were expressed as positive/studied cases.

**Table 8:** Immunophenotyping in 49 cases of Acute Lymphoblastic leukemia by FAB subtype

| Antigen | ALL-L1 | ALL-L2 | ALL-L3 |
|---|---|---|---|
| **CD10** | 9/11 | 33/36 | 2/2 |
| **CD19** | 10/11 | 34/36 | 2/2 |
| **CD20** | 1/11 | 7/36 | 1/2 |
| **CD22** | 9/11 | 28/36 | 2/2 |
| **CD34** | 5/11 | 19/36 | 1/2 |
| **HLA-DR** | 9/11 | 32/36 | 2/2 |
| **CD3** | 1/11 | 2/36 | 0/2 |
| **CD5** | 2/11 | 2/36 | 1/2 |
| **CD7** | 0/11 | 2/36 | 0/2 |
| **CD2** | 0/11 | 1/36 | 0/2 |
| **CD45** | 0/11 | 16/36 | 0/2 |
| **Tdt** | 0/11 | 5/36 | 0/2 |

All values of positive expression were expressed as positive/studied cases.

**Table 9:** Abberant antigen expression in 49 cases of Acute Lymphoblastic leukemia by FAB subtypes

| Antigen | ALL-L1 | ALL-L2 | ALL-L3 |
|---|---|---|---|
| **CD13** | 2/11 | 1/36 | 0/2 |
| **CD33** | 3/11 | 7/36 | 0/2 |
| **CD15** | 0/11 | 2/36 | 0/2 |

All values of positive expression were expressed as positive/studied cases.

**Table 10:** Immunophenotyping in 49 cases of Acute Lymphoblastic leukemia

| Antigen | Pre B ALL | B ALL | T ALL |
|---|---|---|---|
| **CD10** | 35/36 | 9/10 | 1/3 |
| **CD19** | 36/36 | 10/10 | 1/3 |
| **CD20** | 2/36 | 0/10 | 0/3 |
| **CD22** | 35/36 | 10/10 | 0/3 |
| **CD34** | 32/36 | 0/10 | 0/3 |
| **HLA-DR** | 36/36 | 10/10 | 0/3 |
| **CD3** | 0/36 | 0/10 | 2/3 |
| **CD5** | 0/36 | 1/10 | 3/3 |
| **CD7** | 0/36 | 0/10 | 2/3 |
| **CD2** | 0/36 | 0/10 | 1/3 |
| **CD45** | 0/36 | 4/10 | 1/3 |
| **Tdt** | 0/36 | 2/10 | 1/3 |
| **CD13** | 3/36 | 0/10 | 0/3 |
| **CD33** | 7/36 | 0/10 | 0/3 |
| **CD15** | 0/36 | 2/10 | 0/3 |

All values of positive expression were expressed as positive/studied cases.

**Table 11:** Correlation of Immunophenotype to clinical Features of Acute Myeloid and Acute Lymphoblastic Leukemia

| Antigen | Age[a](yrs) | Hba(g/dl) | WBC[a]($10^9$/l) | Plateleta($10^9$/l) | LDH[a] |
|---|---|---|---|---|---|
| **CD3** | 30/28.8 | 10.97/2.9[b] | 204.1/32.2[b] | 105.4/103.4 | 1694.5/1157 |
| **CD5** | 16.8/29.6 | 8.92/8.37 | 198/30.45[b] | 103.8/103.5 | 2851.2/1071.6 |
| **CD7** | 18.1/29.8 | 8.9/8.4 | 117.5/33.3 | 78.5/105.7 | 1582/ 1145.5 |
| **CD10** | 18.9/38.9 | 7.9/8.9b | 33.3/47 | 97.2/109.7 | 1456.6/904.8 |
| **CD19** | 20.2/39.3 | 8.0/8.9 | 32.5/49.5 | 96.2/112.2 | 1508.9/776 |
| **CD20** | 21.6/29.7 | 8.0/8.5 | 13.7/43.3 | 133.3/100 | 764/ 1231.5 |
| **CD22** | 17.2/38 | 7.9/8.8[b] | 27.1/50.6 | 100.8/105.6 | 916.1/ 1397.5 |
| **CD13** | 38.1/22.2[b] | 8.7/8.2 | 31.3/46.6 | 119.2/92.1 | 696.3/ 1534.2 |
| **CD 33** | 37.9/19 | 8.5/8.3 | 34.9/46 | 114.9/91 | 795.7/ 1563 |
| **CD 117** | 37.7/25.8[b] | 8.7/8.3 | 22.2/46.5 | 102.3/103.9 | 584.4/ 1387.5[b] |
| **CD 34** | 30.9/26.8 | 8.3/8.6 | 34/46.6 | 103.1/103.9 | 1036.8/1335.4 |
| **HLADR** | 28.1/32.7 | 8.3/9.0 | 33/77.3 | 95.6/144 | 1221.1/956.2 |
| **CD45** | 31.9/26.5 | 8.6/8.3 | 40.4/40 | 100/106.2 | 837.5/ 1460 |
| **Tdt** | 21.4/29.3 | 9.4/8.4 | 95.1/36.8 | 73.9/105.3 | 852.4/ 1206.6 |
| **CD4** | 40.3/28.3 | 9.8/8.4 | 42.5/40 | 157/100.1 | 432.8/ 1224.3 |
| **CD15** | 25.7/29.1 | 8.5/8.4 | 24.2/41.4 | 68/106.1 | 563.7/ 1235.2 |
| **CD64** | 35.3/28.4 | 8.3/8.4 | 26.5/41.2 | 77/105.5 | 489.8/ 1241.3 |
| **CD11c** | 31.3/28.7 | 7.2/8.5 | 16.4/41.4 | 65.4/105.3 | 304/ 1231.1[b] |
| **MPO** | 32.3/28.7 | 7.8/8.4 | 20.9/40.9 | 80.4/104.3 | 368.7/ 1216.4 |
| **Gly A** | 30/28.8 | 9.4/8.4 | 47.6/39.9 | 78/104.4 | 900.3/ 1195.3 |
| **CD14** | 31/28.8 | 9.4/8.4 | 72.1/39.8 | 99.6/103.5 | 650/ 1191 |
| **CD38** | 53/28.6 | 8.6/8.4 | 1.6/40.6 | 50.8/104.1 | 220/ 1996.6 |

| | | | | | |
|---|---|---|---|---|---|
| **CD61** | 25/29 | 7.4/8.4 | 7.2/41 | 154.5/102.2 | 1805/ 1168 |
| **CD42a** | 2/29.2 | 6.8/8.4 | 13.9/40.5 | 172/102.7 | 3140/ 1159.1 |
| **CD2** | 17/29 | 6.8/8.4 | 340/36.7[b] | 37.4/104.2 | 1180/ 1184.2 |

[a] The mean value of data of positive cases/data of negative cases
[b] $p < 0.05$
The above table shows that the expressions of CD13/CD117, CD3/CD10/CD22, CD3/CD5/CD2 and CD117/CD11c were related to age, Haemoglobin, WBC and LDH levels in peripheral blood respectively. ($p<0.05$)

**Table 12:** Correlation of immunophenotype and abnormal karyotype

| Antigen | Abnormal Karyotypes |
|---|---|
| **CD3** | 0/13 |
| **CD5** | 1/13 |
| **CD7** | 0/13 |
| **CD10** | 7/13 |
| **CD19** | 8/13 |
| **CD20** | 2/13 |
| **CD22** | 6/13 |
| **CD13** | 5/13 |
| **CD 33** | 6/13 |
| **CD 117** | 4/13 |
| **CD 34** | 5/13 |
| **HLA-DR** | 11/13 |
| **CD45** | 4/13 |
| **Tdt** | 0/13 |
| **CD15** | 0/13 |
| **CD64** | 0/13 |
| **CD11c** | 0/13 |
| **MPO** | 0/13 |
| **Gly A** | 0/13 |
| **CD14** | 0/13 |
| **CD38** | 0/13 |
| **CD61** | 0/13 |
| **CD42a** | 0/13 |
| **CD2** | 0/13 |

The values are presented as data of positive cases/data of studied cases.
No significant correlation was noted between the immunophenotype and the cytogenetics.

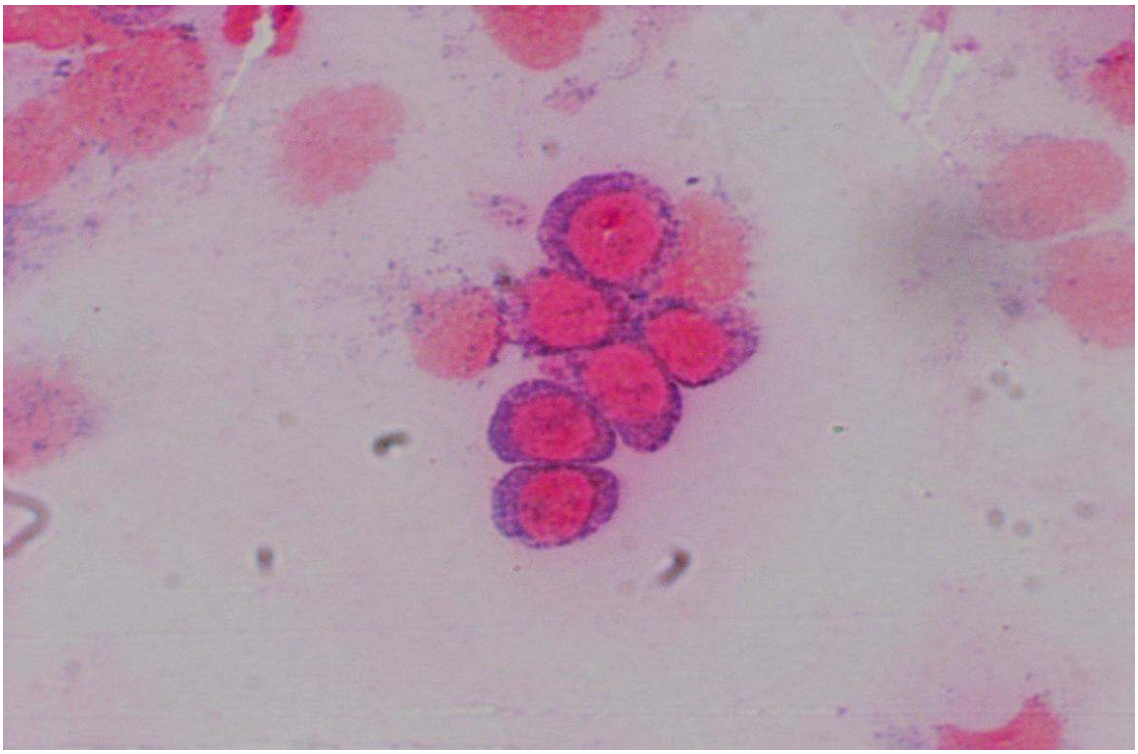

**Figure 1:** MPO positive blasts in AML-M3 (Bone marrow aspirate) 400X

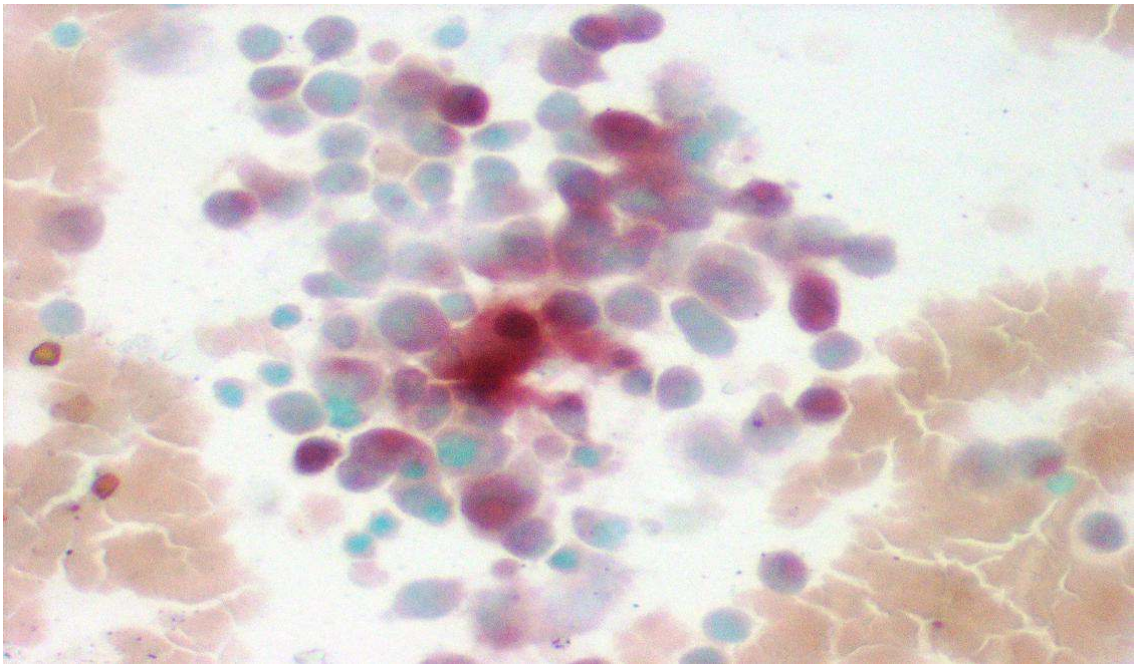

**Figure 2:** Non-specific esterase positive blasts in a case of AML-M5

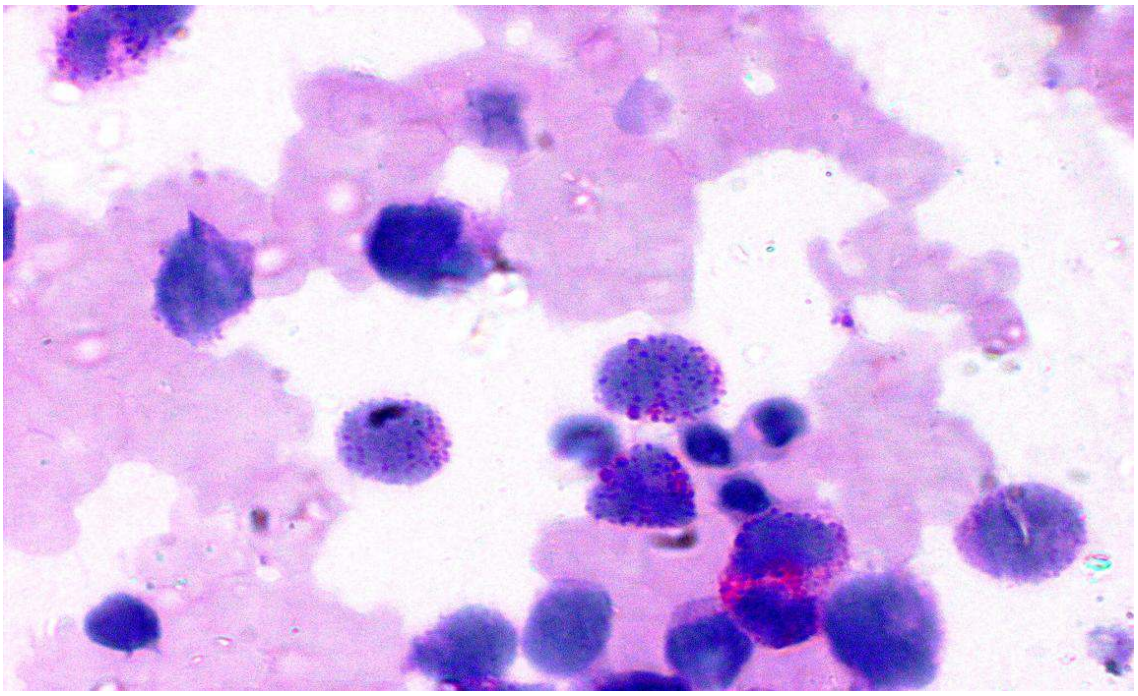

**Figure 3:** PAS positive blasts in a case of AML-M7 (400X)

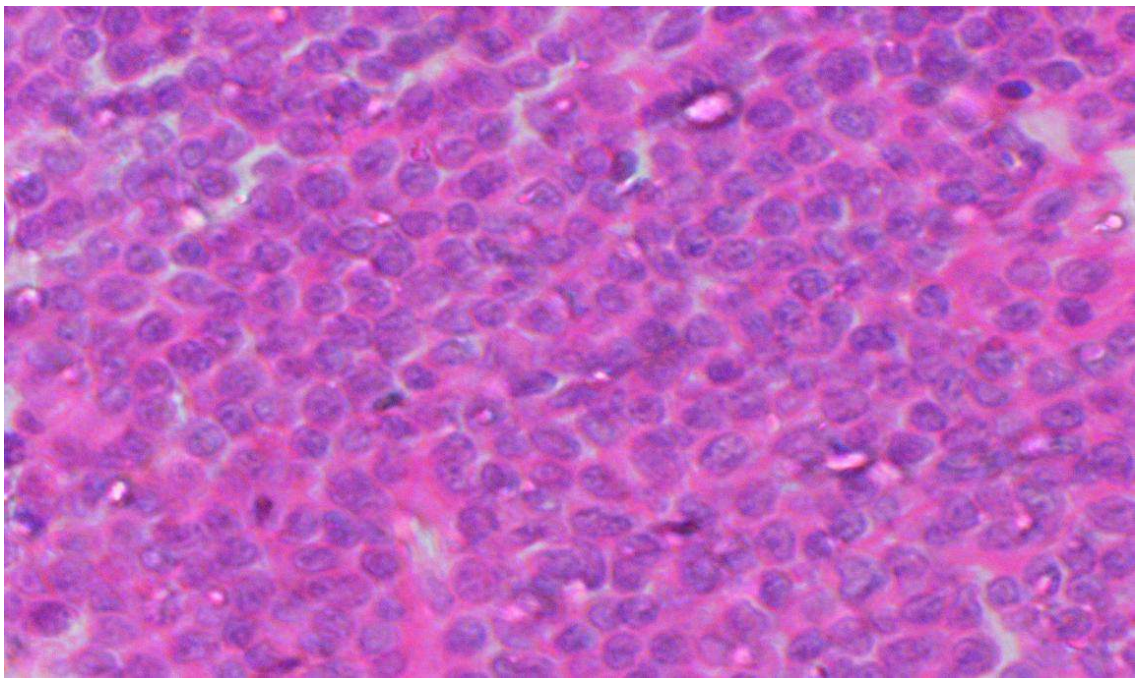

**Figure 4:** ALL blasts in sheets (Bone marrow biopsy) H&E 400X

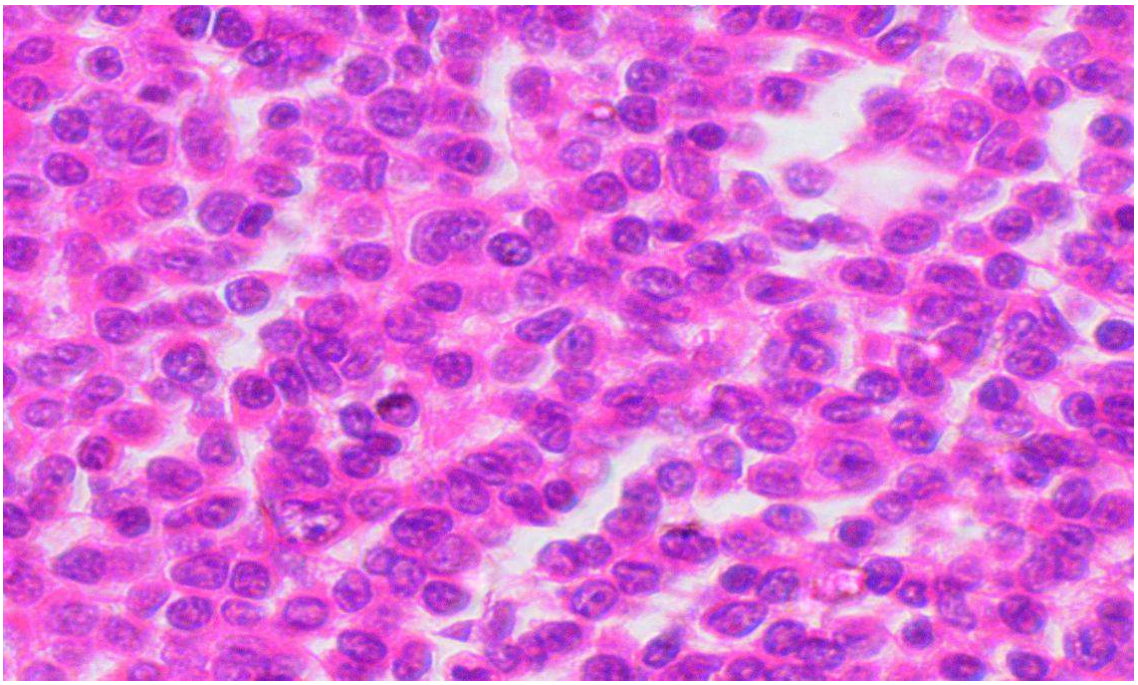

**Figure 5:** Sheets of blasts in a case of AML (Bone marrow biopsy) H&E 400X