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.