## 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.