Original Article

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A Flow-Cytometric Analysis of Spectrum of Acute Myeloid Leukemia at Diagnosis

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ABSTRACT

Background: Acute Myeloid Lymphoma is the clonal proliferation of non-lymphoid blasts comprising at least 20% of total nucleated cells either in bone marrow or peripheral blood. In the recent years, flow cytometry has emerged as a powerful diagnostic tool for AML due to its impact on treatment and prognosis.

Aims & Objectives:

1. To analyse the flow cytometry findings in patients diagnosed as acute myeloid leukemias.

2. To evaluate variations in flow cytometry expression in various subtypes of acute myeloid leukemia

Materials & Methods: Patients diagnosed as acute myeloid leukaemia on peripheral smears were subjected to flow cytometry analysis. This was a four-year study from July 2015 to June 2017 retrospectively and from July 2017 to June 2019 prospectively.

Results: A total of 27 cases diagnosed as Acute Myeloid Leukemia (AML) were included in the study. Acute Promyelocytic Leukemia was observed to be the most common subtype. The most commonly expressed myeloid antigens were CD13 and CD33. There was an aberrant expression of CD7 and CD56 in 1 case each indicating adverse prognosis.

Conclusion: Immunophenotyping of the myeloid cells by flow cytometry has revolutionised the diagnosis of acute myeloid leukemias. It aids in confirming the morphological diagnosis, and also helps in assigning specific lineage, accurate sub classification and adequate treatment in challenging cases. Aberrant expressions were observed in 3 cases of AML. Aberrant antigen expression is associated with a poor outcome. Flow cytometry results interpreted with morphology are not only complementary but also conclusive aiding in therapeutics and predicting prognosis.

Keywords: *Acute Myeloid Leukemia, Acute Promyelocytic Leukemia.*

Introduction

Acute leukemia, being a heterogeneous group hematopoietic neoplasm presents with diverse clinical, morphological and antigenic profile. [1] Acute myeloid leukemia (AML) is a malignant disease of the bone marrow in which hematopoietic precursors are arrested in an early stage of development. Most AML subtypes are distinguished from other related blood disorders by the presence of more than 20% blasts in the bone marrow. The French-American-British (FAB) introduced in 1970 categorised AML into eight subtypes M_0-M_7 based on morphology and cytochemistry. [2,3] Owing to the pitfalls of FAB classification, it was replaced by World Health Organisation (WHO) classification which categorises acute leukemia based on Immunophenotyping and cytogenetic abnormalities. [4]

Immunophenotyping of the neoplastic cells in the peripheral blood and bone marrow by flow cytometry (FCM) has become mandatory in classifying leukemia in the recent years due to prognostic implications. [5] It provides an insight into identifying the lineage of leukemic cells and their pathway of differentiation. [6] It enables identifying minimal residual disease and relapse cases accurately even when the counts are low. [7] Expression of aberrant antigens in AML is commonly associated with a poor outcome. Assessment of antigenic profile and cytogenetic abnormalities is imperative as it plays an important role in prognosis and treatment response. FCM thus helps in identifying acute myeloid leukemias with better precision. [8]

Aims & Objectives

1. To analyse the flow cytometry findings in patients diagnosed as acute myeloid leukemias.
2. To study the immunophenotypic expression in various subtypes of acute myeloid leukemia.

Materials and Method

Source of data: The present study was conducted on 27 bone marrow aspirate and biopsy specimens received at the tertiary care center.

Duration of the study: The total duration of the present study was four years. Two-year retrospective study was done from July 2015 to June 2017 and two-year prospective study from July 2017 to June 2019.

Inclusion criteria: The following samples received in the Department of Pathology, A.J Institute of Medical Sciences and Research Centre, Mangalore will be included for the study.

1. All samples of patients irrespective of age and gender
2. All samples of patients diagnosed as acute myeloid leukaemia on peripheral smear.

Exclusion criteria

1. Inadequate samples or poorly preserved samples are excluded.
2. Post chemotherapy and stage 4 lymphoma patients are excluded.

Methodology

1. The age of the patient, gender, presenting symptoms and physical examination findings were documented.
2. Peripheral vein blood sample was collected from the patients in EDTA test tubes.
3. Peripheral blood smears were stained with Leishman's stain and examined. Cytochemical stains used were Sudan black B and Periodic acid Schiff stain.
4. Bone marrow aspirate and biopsy sample was taken from posterior superior iliac spine using gauge needle for adults and gauge needle for paediatric patients.
5. Smears prepared from bone marrow aspirate were stained with Leishman's stain and examined. Special stains used were Periodic acid Schiff and Perl's stain.
6. Paraffin embedded bone marrow biopsy sections were stained with haematoxylin and eosin stain and examined.
7. For flow cytometry studies samples were collected in heparin vacutainers.
8. The instrument used for flow cytometry analysis was BD FACS Canto II and software used was BD FACS DIVA.
9. The staining method used was stain lyse wash method followed by gating with SSC versus CD45.

Results and Analysis

A total of 27 cases were included in the present study. The most common subtype of AML in the present study was acute promyelocytic leukemia (APL), the total number of cases being 8. Only 1 case was diagnosed as AML with inv(16). [Figure-1]

In this study, males were found to be more commonly affected than females. The total number of male patients diagnosed with AML was 14 and female patients were 13. The male to female ratio was 1.07:1. The age range in the present study of 50 cases was from 4 to 79 years. The mean age was 38.6 years.

Majority of cases were APML followed by AML M2 and AML M4. Also, APML was found to predominantly affect the male patients. Correlation of AML cases with flow cytometry was found to be in 96.4 % of the cases.

The most common type of AML in this study was APL which showed expression of CD13, CD33, CD45, CD117 and MPO. AML with inv(16) showed expression of HLA DR, CD34, CD13, CD33, CD117, cMPO and CD15. Three cases which were not subtyped on flow cytometry showed expression of CD34, CD33, CD117, CD13, HLA DR, MPO, CD38 and CD45.

Two cases of AML showed aberrant expression of CD7 (one case each of AML M0 and AML M2) and one case of AML M2/M4 showed aberrant CD56 expression. [Table-1]

Table 1: Antigens expressed in AML.

AML Subtype	Antigens expressed	Antigens notexpressed
AML M0	CD13, CD33, CD45, CD34, HLA-DR, CD117, CD38	CD64, MPO
AML M2	CD13, CD33, CD65, CD11b, CD15	HLA-DR, CD14, CD64
AML M4	CD34, CD33, CD38, CD64, CD36, CD11b, CD13, HL4ADR, CD117, MPO, CD14	CD16, TdT
AML M2/M4	CD33, CD11b, CD117, CD45, CD38, CD56, MPO, CD13	CD14, CD64, CD34
APL	CD13, CD33, CD45, CD117, MPO	HLA-DR, CD34
AML with inv(16)	HLA DR, CD34, CD13, CD33, CD117, cMPO, CD15	CD11b, CD14
AML Unclassified	CD34, CD33, CD117, CD13, HLADR, MPO, CD38, CD45	-

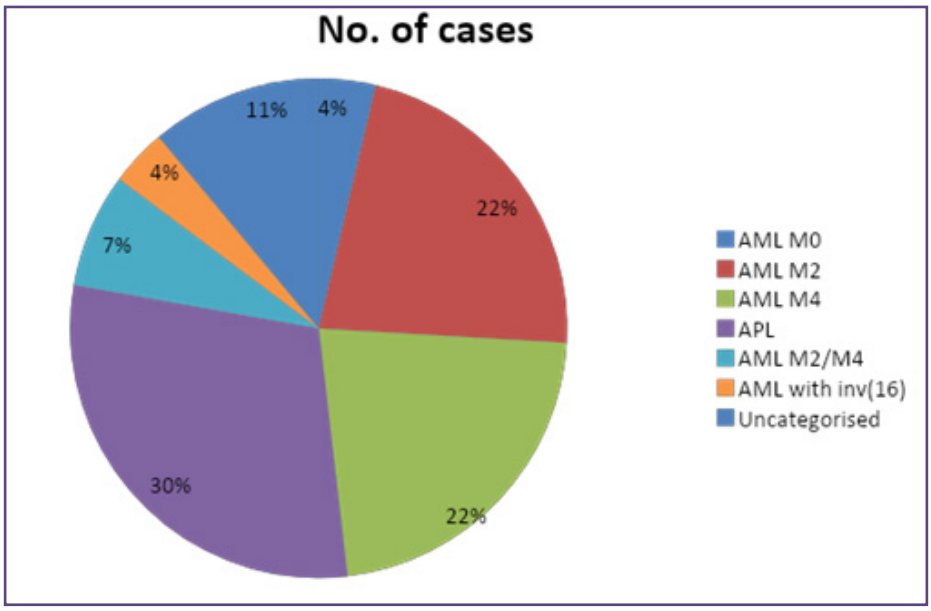
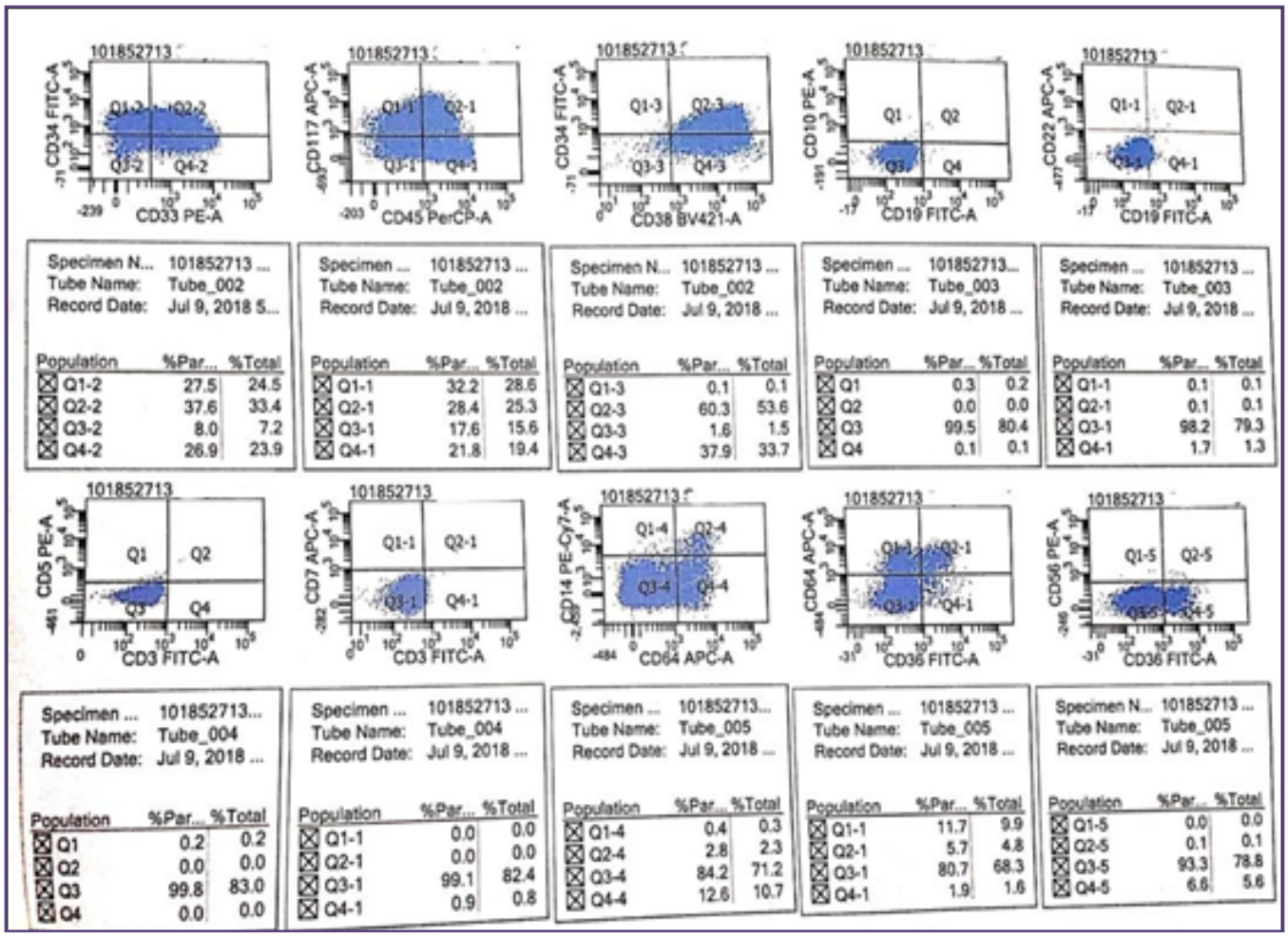


Fig. 1: Distribution of AML subtypes.



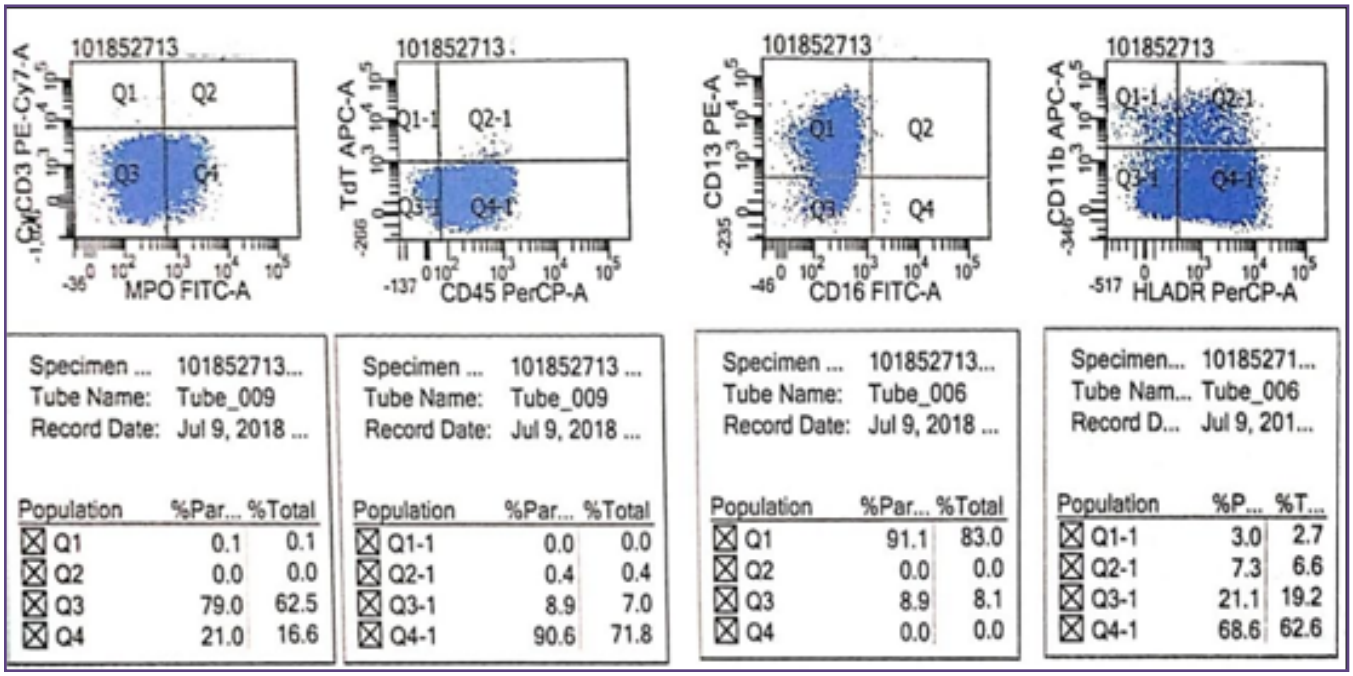


Fig. 2: Immunophenotyping by FCM in AML M2/M4.

Discussion

In the present study conducted at Central Diagnostic Laboratory of AJ Institute of Medical Sciences, Mangalore, twenty seven of acute leukaemia were analysed. The mean age of occurrence of acute myeloid leukaemia in the present study was 38.6 years. The highest number of cases was observed in the age group of 41-60 years. Overall males were affected more commonly than females.

Acute Myeloid Leukaemia

Majority of the cases were observed in the age group of 41-60 years. Whereas literature by Horner MJ et. al says >50% of the cases occur at the age of more than 65 years. [9] The incidence was seen to be almost equal among both males and females with a male to female ratio of 1.07:1.

Immunophenotyping is done to differentiate between myeloid and lymphoid precursor cells. Majority of AML cases show reactivity for CD13, CD15, CD33, CD64 and CD117.

Recently, karyotyping has been suggested for most of the cases as it plays a crucial role in appropriate classification and determination of prognosis. [3,10] The distinct features of each category under AML have been described below.

On flow cytometry, AML M0 leukemic cells showed moderate expression of CD34, CD38 and HLA-DR, dim expression of CD13, CD33, CD117 and CD45. Also, there was aberrant expression of CD7 in this case. A study done by Poeta et al. showed that CD7 positive AML cases had reduced survival rate. [11] These cells do not show either morphological or cytochemical characteristics of myeloid differentiation. Immunophenotyping shows the majority of these cells express CD32, CD38 and HLA-DR positivity. They may also show positivity for CD33, CD13, CD117 and Tdt. [3,12]

The total number of cases diagnosed as AML M2 in this study was six (22%) which was the second most common subtype whereas Ghosh et al found AML M2 as the most common subtype in their study. [12] Flow cytometry analysis of AML M2 showed leukemic cells expressing CD13, CD33, CD65, CD11b and CD15. Study conducted by Poeta GD et. al also showed AML immunophenotypically commonly show positivity for CD13, CD33, CD65, CD11b, and CD15 and CD34, CD117 and HLA-DR may be positive only in a few cases. [3,11]

With AML M4 all the cases showed bright expression of HLA-DR, moderate expression of CD33, CD117 and CD13, dim expression of CD34, CD45, CD38, and MPO.

Two cases (7%) were given a final diagnosis of AML M2/M4 on flow cytometry. They showed moderate expression of CD33, CD11b, dim expression of CD117, CD45, CD38, MPO, heterogeneous expression of CD13. In one case, aberrant CD56 expression was detected. Alegretti AP et al. demonstrated that expression of CD56 was most commonly found in AML M4 subtype. [8] A study done by Chang H et al. back in 2004 showed that CD56 expression in AML was associated with poor survival rate, reduced

response to chemotherapy and increased occurrence of CNS involvement. [14]

Acute Promyelocytic Leukaemia on FCM, was negative for CD34. All the cases showed moderate expression of CD33, CD13 and MPO, dim expression of CD45 and CD117 on flow cytometry analysis. In seven cases cytogenetic analysis showed PML-RARA translocation. Immunophenotypically they express myeloid lineage markers along with weak or absent HLA-DR and absent CD34. In hypogranular variant, the nucleus is folded with indistinct granules and CD34 is positive. The cases which are negative for FLT3 have poorer prognosis. Lee J J et. al. in a study say that treating these patients with All Trans Retinoic Acid (ATRA) with or without anthracycline has helped to attain complete remission. [3,15]

AML with inversion (16) on flow cytometry the antigens expressed were HLA DR, CD34, CD13, CD33, CD117, cMPO and CD15 but lacked expression of monocyte lineage markers CD14, CD11b, CD11c and CD64. FISH analysis done on the patient's sample was positive for inv(16) CBF Beta gene rearrangement.

Three cases (11%) diagnosed as AML based on peripheral smear and bone marrow findings could not be subtyped even on flow cytometry. The antigens expressed in these cases were CD34, CD33, CD117, CD13, HLADR, MPO, CD38 and CD45.

In one case (4%) the morphological diagnosis was given as AML with multilineage dysplasia. However, the final diagnosis on flow cytometry was MDS-RAEB 2.

Out of 27 cases diagnosed on morphology as AML, 26 cases (96.4%) correlated with flow cytometry diagnosis of AML. Flow cytometry analysis was not available in 4 cases diagnosed as AML morphologically as the patients refused for further evaluation or were referred to a higher centre for treatment.

Conclusion

Flow cytometry analysis of acute leukemia has become a standardised technique in the recent days for categorizing the cases as emphasized by 2016 WHO classification. Majority of cases were acute myeloid leukemia occurring in individuals above 40 years with a male predominance. It was also observed that acute promyelocytic leukemia (APL) was the most common subtype. Prompt diagnosis of APL is important as it can present with coagulation abnormalities and also has a favourable prognosis when treated with all trans retinoic acid (ATRA). Aberrant expression of CD7 was observed in two cases and CD56 in one case of AML. Aberrant antigen expression is associated with a poor outcome.

We observed a significant correlation between morphological and flow cytometry analysis. Hence FCM helps in confirming the morphological diagnosis, assigning specific lineage to unclassifiable cases and detection of aberrant antigens which is necessary to predict treatment response and patient survival. It is pertinent to conclude that flow cytometry results interpreted with morphology are not only complementary but also conclusive aiding in therapeutics and predicting prognosis.

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Competing interests

None declared

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