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Original Articles

Immunophenotypic Characterisation Along with Aberrant Expression of CD markers in Morphologically Diagnosed Cases of Acute Myeloid Leukemia

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Abstract

Immunophenotyping of leukemia cells is useful for detecting leukemia cell line, determining maturation stage and identifying aberrant antigens which act for individual treatment monitoring and detection of residual disease. A total of 104 newly diagnosed cases of acute myeloid leukemia were identified at hematology department in National Institute of Cancer Research and Hospital from January 2020 to December 2021. We detect Immunophenotypic pattern in newly diagnosed cases of acute myeloid leukemia. We also determine the frequency and pattern of aberrant expression of CD markers in acute myeloid leukemia patients. Mean age of patients was 35 years (SD±16 years) with male to female ratio was 1.53:1. Most frequent morphologic subtype was AMLM2 constituting 33.6% of all AML cases. Lineage specific markers HLADR, CD13, CD33, MPO, CD117 and CD34 were expressed in 80%, 89%, 95%, 77%, 74% and 62% cases of all AML cases respectively. Among 104 AML patient, aberrant CD expression was observed in 36% cases. The most frequently observed aberrant markers were CD7 and CD19 lymphoid markers, that were expressed in 15.38% and 14.42% cases respectively. Less frequent aberrant cCD3, CD10, CD5 and cCD79a antigens were expressed in 2.88%, 1.92%, 0.96% and 0.96% cases respectively. Immunophenotyping is essential in diagnosis and sub-classification of AML and expression of aberrant CD antigens is common in acute myeloid leukemia. These findings suggest the necessity for a more extensive study to evaluate the prognostic significance of aberrant CD marker expression in AML and to improve the accuracy of diagnosis and classification of AML.

Keywords: *Acute myeloid leukemia, immunophenotyping, aberrant expression, CD markers.*

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Introduction

Acute Myeloid Leukemia (AML) is a clonal disorder of haemopoietic stem cells characterized by clonal proliferation of immature myeloid cells at various stages of maturation.[1,2] Leukemia is diagnosed by morphology and cytochemical examination of blast cells, immunophenotyping, cytogenetic and molecular genetics.[3] For the diagnosis of hematological malignancy, immunophenotyping is mandatory.[4] The prognosis and survival of adult AML patients remain low, compared with other hematologic malignancies, despite technological advances. Immunophenotyping has a key role in the pathophysiology, prognosis, and overall survival (OS) rate of AML patients[5] and in identification of aberrant antigens which serves for individual treatment monitoring and detection of residual disease. Several immunophenotypes of blast cells from patients of acute leukemia lack normal cellular differentiation and show unusual expression of CD markers called aberrant expression of markers. However aberrant CD markers expression has been observed in several cases of acute leukemia.[6-8] AML has a wide range of heterogeneous immunophenotypic characteristics which is most likely due to genetic diversity. [9] Detection of recurrent genetic abnormalities has priority in the classification of AML.[10,11]

Previous studies found that a large number of antigens influence AML prognosis and prediction. Despite this, reliable risk categorization for diagnosis based on immunophenotypic characteristics continues to be a challenge.[12] Improving the accuracy of prognostic assessment of AML may allow for more targeted and risk adapted treatment, increasing the likelihood of cure and reducing treatment related morbidity and mortality.[13]This study was conducted to detect Immunophenotypic pattern and to determine the frequency and pattern of aberrant expression of CD markers in acute myeloid leukemia patients.

Methods

This is a descriptive type of study and was conducted from January 2020 to December 2021 in National Institute of Cancer Research and Hospital (NICRH). Patients of both genders and all age groups of De novo acute myeloid leukemia were included in the study. A total of 104 patients aged 12 to 80 years were included in this study. Relapsed cases of AML and those evolving from myelodysplasia (MDS) or receiving treatment for AML were excluded. Data were collected at pre-designed datasheet.

Sample Collection and Preparation

Two and a half ml of venous blood in Ethylene Diamine Tetra Acetic Acid (EDTA) tube was collected for complete blood count (CBC) and peripheral blood film examination, under aseptic conditions. Bone marrow aspiration was collected from 104 patients and done after written informed consent, following standard guidelines. Bone marrow aspiration was performed by experienced personnel of the hematology department of NICRH from posterior superior iliac spine after ensuring strict asepsis and necessary precautions. Leishman stained smears from peripheral blood and bone marrow aspirates were examined under microscope for morphology and percentage of blasts. Complete blood counts were generated using automated hematology analyzer Sysmex KN-550.

Methods for immunophenotyping

Sample collected in EDTA tube was immediately transported to different specialized laboratory of Dhaka including Bangabandhu Sheikh Mujib Medical University, Armed Forces Institute of Pathology, International Centre for Diarrhoeal Disease Research, Bangladesh and Evercare Hospital, Dhaka for immunophenotyping. Measured amount of sample (2-5ml) was taken in previously marked tubes to ensure approximate cell concentration of 10^6 ml. Immunophenotyping was done within 48 hour of sample collection. Flow cytometric analysis was made by BD FACS Canto multicolor flow cytometer using standard panel on peripheral blood or bone marrow samples. The surface and cytoplasmic antigens of interest were analysed and correlated with morphological findings.

Result

A total of 104 patients aged 12 to 80 years were included in this study. The mean age of patient was 35 years with standard deviation (SD) of 16 years. Male patient was 60.57 % and female patient was 39.43 %. Male to female ratio was 1.53:1. Most of the female patients were house wife (35.58%), 19.23 % patients were student, 7.1% patients were farmer, 11.54 patients were day laborer, 14.42 % patients were service holder and 12.13% were businessman. 75 patients were married and 29 patients were unmarried (Table 1).

Table-1. *Patient characteristics of study cases:(n:104)*

Demographic Characteristics		
Age in years; mean±SD		35±16
Gender; n (%)	Male	63 (60.57%)
	Female	41 (39.43)
Education; n (%)	Illiterate	23 (22.12)
	Undergraduate	60 (57.69)
	Graduate	21 (20.19)
Occupation	Student	20 (19.23)
	Housewife	37 (35.58)
	Farmer	7.38 (7.10)
	Day laborer	12(11.54)
	Service holder	15 (14.42)
	Business man	12.62 (12.13)
Marital status	Married	75 (72.11)
	Unmarried	29 (27.89)

Table 2. *Complete blood count of newly diagnosed AML cases*

Blood parameters	Mean ±SD	Highest level	Lowest level
Hemoglobin level in gm/dl	7.45 ± 2.35	13.8	2.1
Total WBC count x 10^9/L	52.19 ± 67.96	365.00	0.76
Total Platelet count x10^9/L	52.00± 50.19	260.00	4.00

WBC= White blood cell, SD = Standard deviation

Mean haemoglobin level was 7.45±2.35 gm/dl, the mean TLC was 52.19±67.96 x 10^9 /L, lowest total leucocyte count was 0.76 x 10^9/L and highest total leucocyte count was 365.00 x 10^9/L, mean of platelet count was 52.00±50.19 x 10^9/L, lowest platelet count was 4.00 x 10^9 /L and highest platelet count was 260.00 x 10^9 /L (Table 2).

In peripheral blood, the average number of blast cells was 55% (range from 0% to 96%). In bone marrow, highest number of blast cells was 96% and lowest number of blast cells was 20%. Most frequent subtype was AMLM2 constituting 35 (33.6%) cases followed by AMLM1 (n=18, 17.3%) and least common was AMLM7 (n=2, 1.9%).

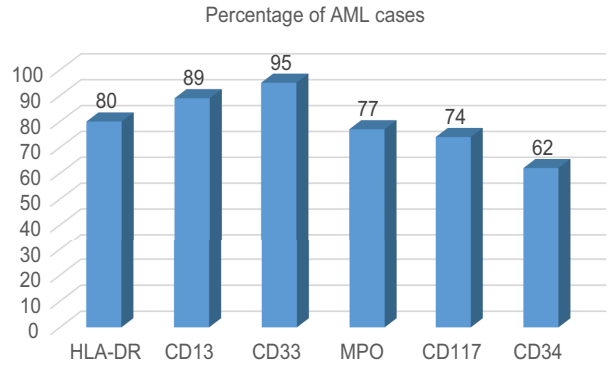

Figure 1: *Percentage of expression of myeloid CD markers in acute myeloid leukemia patients*

CD45 was positive in all cases. Among 104 cases HLADR was expressed in 83 (80%) cases, CD13 was positive in 93 (89%) cases, CD33 was positive in 99 (95%) cases, MPO was positive in 80 (77%) cases, CD117 was positive in 77 (74%) cases and CD34 was positive in 65 (62%) cases (Figure 1).

Table 3: CD antigen expression in acute myeloid leukemia

Cases	n	%
Lineage specific CD expression	67	64%
Aberrant CD expression	37	36%

CD = Cluster of differentiation, n: Number, %: Percentage

Among 104 AML cases 64% cases (n=67) have lineage specific CD markers expression and 36% cases (n=37) have aberrant CD markers expression (Table 3).

Table 4. *Aberrant CD expression in 37 acute myeloid leukemia cases.*

Aberrant CD expression	n	%
CD7	16	15.38
CD19	15	14.42
cCD79a	1	0.96
CD10	2	1.92
CD5	1	0.96
CD56	9	8.6
cCD3	3	2.88
CD7+CD5	1	0.96
CD7+cCD3	1	0.96
CD7+CD10	1	0.96
CD10+CD19	1	0.96
CD19+CD56	**4**	**3.84**

CD = Cluster of differentiation, n: Number, %: Percentage

CD7 was the most frequent aberrant antigen and was positive in 16 (15.38%) cases, CD19 showed positivity in 15 (14.42%) cases, CD5 showed positivity in 1 (0.96%) case, cCD3 showed positivity in 3 (2.88%) cases, CD79a showed positivity in 1 (0.96%) case, CD10 showed positivity in 2 (1.92%) cases and CD56 showed positivity in 9 (8.6%) cases. Two antigens CD5 and CD7, cCD3 and CD7, CD10 and CD7, CD10 and CD19, CD56 and CD19 were expressed in 1,1,1,1 and 4 cases respectively (Table 4).

Discussion

Multiparameter flow cytometry (MFC) immunophenotyping is an important technique in the diagnosis, classification and monitoring of AML.[5] In this study, the mean age of patient was 35±16 years. Mean age of patient was 31.81 years with SD of 19.972 years in another study.[2] In our study

male patient was 60.57 % and female patient was 39.42 %. Males constituted 58.5% and females were 41.50% by Basharat at al.[2] In this study male to female ratio was 1.53:1. The male to female ratio was 1.4:1 in another study.[2] In our study, mean haemoglobin level was 7.45±2.35 gm/dl. The mean haemoglobin level was 8.25±2.16 g/dl in another study.[2] The mean TLC was 52.19±67.96 x 10^9 /L, lowest total leucocyte count was 0.76 x 10^9/L and highest total leucocyte count was 365.00 x 10^9/L (shown in table 1), in this study. The mean TLC was 57.46±79.39 x 10^9/L, the lowest being 0.99 x 10^9 /L and highest being 456.77x10^9/L both extremes seen in AML-M1 by Basharat at al.[2] In our study, mean of platelet count was 52.00±50.19 x 10^9/L. Lowest platelet count was 4.00 x 10^9 /L and highest platelet count was 260.00 x 10^9 /L (shown in table1). The mean of platelet count was 58.23±81.29 × 10^9 /L, the highest count was 1000 ×10^9 /L seen in AML-M2 and lowest being 659 × 10^9 /L seen in AML-M1 by Basharat at al.[2] In peripheral blood, the average number of blast cells was 55% (range from 0% to 96%). In bone marrow, highest number of blast cells was 96% and lowest number of blast cells was 20%, in this study. In another study, the least number of blasts were 22% seen in AML-M2 and AML-M6 and highest abnormal promyelocytes were 98 %, found in AML-M3.[2]

In this study, most frequent subtype was AMLM2 constituting 35 (33.6%) cases followed by AMLM1 (n=18, 17.3%) and least common was AMLM7 (n=2, 1.9%). M1-2 was the most frequent AML subtype, according to most published data.[14-18]AMLM2 was the most frequent subtype constituting 49 (47.2%) cases followed by AML-M3 (n=18,17%) and the least common being AML-M6 (n=3, 2.8%) by Basharat at al.[2]

All cases CD45 was positive. Among 104 cases HLADR was expressed in 80% cases, CD13 was positive in 89% cases, CD33 was positive in 95% cases, MPO was positive in 77% cases, CD117 was positive in 74% cases and CD34 was positive in 62% cases (shown in figure 1), in this study. HLA-DR was expressed in 89.6% of AML (non-APL) cases, CD34 showed mean positivity of 62.1% among all AML subtypes, CD33 was positive in 89.4% cases, CD13 was positive in 77.9% cases, CD117 was positive in 74.3% cases of all AML subtypes by Salem at al.[14] MPO has an important role in distinguishing minimally differentiated AML (M0) and biphenotypic acute leukemia from acute unclassified leukemia and acute lymphoblastic leukemia. MPO-negative AML cases consisted of M0 and M5 according to the FAB classification.[19]

Among 104 AML cases 64% cases (n=67) have lineage specific CD markers expression and 36% cases (n=37) have aberrant CD markers expression (shown in table 2), in our study. This result is consistent with the findings of other studies. Among 23 AML cases, 57% cases had lineage specific CD markers and 43% cases were shown to express aberrant CD markers by Chughtai at al.[6] Aberrant phenotypes were found in 21 (58.3%) cases of AML in one study[3] and in 28 (58%) cases of AML in another study.[20] Identification of aberrant antigens has significant role for individual treatment monitoring and detection of residual disease after completion of chemotherapy in AML patient.[6-8]

In our study, CD7 was the most frequent lymphoid associated antigen found in AML and was positive in 16 (15.38%) cases, CD19 showed positivity in 15 (14.42%) cases, CD5 showed positivity in 1 (0.96%) cases, cCD3 showed positivity in 3 (2.88%) cases, CD79a showed positivity in 1 (0.96%) cases, CD10 showed positivity in 2 (1.92%) cases and CD56 showed positivity in 9 (8.6%) cases. Two antigens CD5 and CD7, cCD3 and CD7, CD10 and CD7, CD10 and CD19, CD56 and CD19 were expressed in 1,1,1,1 and 4 cases respectively (shown in Table 3).

According to the published data, CD7 is the most frequent lymphoid associated antigen that is aberrantly expressed in AML. The aberrant expression of CD7 and CD56 in AML patient is associated with poor outcome and shorter overall survival.[21] The aberrant expression of CD7 showed positivity in 23% AML cases in one study[14] and in 6 (26%) AML cases in another study[6] and in 7 (25%) AML cases in other study.[20] CD19 was expressed in 1.8%AML cases in one study[14] and 1 (4%) AML case in another study.[6] CD19 expression in AML cases warrants the search for specific cytogenetic defect because aberrant expression of CD19 in AML is commonly associated with t(8;21).[22] CD5 was expresses in 5 (16.7%) cases in one study[23] and 3(13%) cases in another study.[6] CD3 was expressed in 3 (10%) cases in one study[23] and 1(4%) case in another study[6] and 8 (29%) cases in other study.[20] CD56 was expresses in 8 (16.7%) cases of AML in the study of Alegretti at al. The expression of CD56 in AML may associated with poor prognosis and shorter overall survival.[24] Pinheiro at al showed that aberrant expression of CD7, CD56, CD15, CD2, CD3, CD90, CD123, CD117 in AML had shown negative impact on prognosis and three others; CD19, CD98 and CD117/CD15 were associated with good prognosis.[25]

So we concluded that almost all myeloblasts expressed CD45 and other most commonly expressed antigens are CD13, CD33, MPO, CD117 and HLA-DR. Aberrant phenotypes are present in a large proportion of AML cases and CD7, CD19 are the most common aberrant antigens that are expressed in AML.

Conclusion

Diagnosis of AML is improved by flow cytometric analysis of acute leukemia using a combination of patterns and intensities of antigen expression. In acute myeloid leukemia, presence or absence of aberrant CD antigen markers indicates a poor or good prognosis. As a result, it is important to know if there is any aberrant CD antigen expression in acute myeloid leukemia in order to anticipate the disease prognosis. However, further prospective studies with larger sample sizes and extended panel of monoclonal antibodies in MFC routine of acute myeloid leukemia are needed.

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