



Routine Diagnostic Immunophenotypic Profiling in AML Identifies Chemo Resistant Patients and Predicts Treatment Outcomes

Birva Raiya ^a, Shana Laliwala ^a, Jayendra Patel ^b,
Pina Trivedi ^c and Hemangini Vora ^{a*}

^a Immunohematology Lab, The Gujarat Cancer and Research Institute, Ahmedabad, India.

^b Molecular Diagnostic and Research Lab 2, The Gujarat Cancer and Research Institute, Ahmedabad, India.

^c Cytogenetic Lab, The Gujarat Cancer and Research Institute, Ahmedabad, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/123475>

Original Research Article

Received: 21/07/2024

Accepted: 23/09/2024

Published: 27/09/2024

ABSTRACT

Aim: This study aimed to examine different phenotypic stages of arrest observed in Acute Myeloid Leukemia.

Materials and Methods: A total of 220 patients with Acute Myeloid Leukemia (AML) were categorized into five subgroups Hematopoietic stem cells (HSCs), Multipotent Progenitors (MPPs), Common Myeloid Progenitors (CMPs), Common Myeloid Progenitors (CMPs), Granulocyte/Macrophage Progenitors (GMPs) based on their pattern of stage of leukemia arrest

*Corresponding author: E-mail: hemangini.vora@gcriindia.org;

Cite as: Raiya, Birva, Shana Laliwala, Jayendra Patel, Pina Trivedi, and Hemangini Vora. 2024. "Routine Diagnostic Immunophenotypic Profiling in AML Identifies Chemo Resistant Patients and Predicts Treatment Outcomes". *Asian Hematology Research Journal* 7 (4):160-69. <https://www.journalahrj.com/index.php/AHRJ/article/view/180>.

(SLA) using an Immunophenotypic profile with routine myeloid markers (MPO, CD13, CD33, CD14, HLADR, and CD34) at diagnosis and were correlated with clinicohematological parameters, molecular and cytogenetic characteristics, and disease status.

Results: The distribution of the SLA phenotypes showed 21% with MPP, 26% with CMP, 21% with GMP, and 37% with GP/MP and none of the patient showed HCP phenotype. In relation to clinicohematological parameters, male patients had maturation arrest at an earlier stage of differentiation (MPP/CMP), whereas female showed in a later stage (GP/MP). High white blood cell (WBC) and blast counts exhibited maturation arrest at the later stage (GP/MP). In relation with FAB subtypes, 83% AML-M3, 42% of AML-M4, and 50% of AML-M5 are associated with the GP/MP phenotype, while 67% of AML-M0 and 23% of AML-M1 are associated with the MPP phenotype. Further, maturation arrest at the MPP level was associated with aberrant CD7 expression. In relation with mutations in FLT3-ITD/ FLT3 D835 were associated with MPP (27%) and CMP (40%), C-kit mutations with CMP stage (67%), whereas NPM1 mutations (75%) and CEBPA mutations (50%) were showed arrest at the progenitor stage (GP/MP). In relation to treatment response, 75% with the MPP phenotype, 13% with CMP, 27% with GMP, and 19% with GP/MP showed persistent leukemic activity. This suggests that the MPP group is the most resistant to chemotherapy.

Conclusion: Acute Myeloid Leukemia Immunophenotyping can identify a new SLA phenotype that effectively correlates with leukemic cell behavior and helps predict major genetic subgroups at diagnosis, along with their response to standard intensive chemotherapy.

Keywords: Immunophenotyping; AML; stage of leukemia arrest; chemo resistance.

1. INTRODUCTION

Leukemia is characterized by abnormal cell differentiation leading to an increased number of blast cells with impaired functionality. It constitutes approximately 5% of all human cancers, with about half of these cases categorized as Acute Leukemia (AL). Acute leukemia includes both Acute Lymphoid Leukemia (ALL) and Acute Myeloid Leukemia (AML), affecting an estimated 4 million individuals annually in developing countries [1,2].

During normal hematopoiesis, precursor cells originate from Hematopoietic Stem Cells (HSCs). These cells have the capacity for indefinite self-renewal and differentiate into non-self-renewing Multipotent Progenitors (MPPs) which loses self-renewal capacity but differentiate in to precursor cells. From MPPs, two main branches emerge: Common Lymphoid Progenitors (CLPs), which finally differentiated into T lymphocytes, B lymphocytes, and natural killer (NK) cells; and Common Myeloid Progenitors (CMPs), which differentiate into Granulocyte/Macrophage Progenitors (GMPs) responsible for producing monocytes, macrophages, and granulocytes, and Megakaryocyte/Erythroid Progenitors (MEPs) that generate megakaryocytes, platelets, and erythrocytes (Red blood cells) [3-7].

Precursor cells (blast cells) from in Acute Myeloid Leukemia, express cell surface markers (CD markers) specific to their developmental stage.

These markers serve as phenotypic indicators used to identify the stage of cell differentiation arrest and Multiparametric Flow cytometry is helpful to characterize these blasts in Acute Myeloid Leukemia patients, resembling the organized structure seen in normal blood cell formation [7-11].

In this study, we conducted an analysis of a AML patients to investigate their phenotype arrest at diagnosis. Our goal was to identify distinct AML surface profiles influenced by the stage of leukemia arrest (SLA). We assumed that understanding of leukemic arrest could help in existing AML classifications by identifying unique phenotypic subtypes linked to specific clinical and molecular features. Furthermore, we aimed to gain insights into treatment outcomes following standard therapies. Ultimately, integrating this phenotypic classification with morphological analysis available within 24 hours of diagnosis could greatly facilitate the planning of therapeutic strategies in everyday clinical settings.

2. MATERIALS AND METHODS

2.1 Study Design

This retrospective study was conducted at the Immunohematology Laboratory, The Gujarat Cancer and Research Institute (GCRI), Ahmedabad, involving 220 patients diagnosed with AML between January 2022 and May 2024.

2.1.1 Patient characteristics and variables

All 220 patients were diagnosed as Acute Myeloid Leukemia through morphological examination along with cytochemistry and Flow-cytometric Immunophenotyping expression. Hematological data, including WBC count, FAB subtypes, gender, age, clinical outcomes, were retrieved from the Institute's records Department. Cytogenetic and Molecular testing were carried out by Cytogenetic and Molecular diagnostic laboratories of within the institute and data were subsequently correlated. Out of 220 patients, detail treatment history was available in 175 patients, of which 73 patients has received standard chemotherapy with Ara-C and had follow-up to 24 months.

2.2 Immunophenotyping of AML

Multi-parameter flow cytometry (MFC) was conducted with standardized 8-color EURO Flow panel on either bone marrow (BM) or blood samples (1×10^5 cells per tube) using a standard stain-lyse-wash (BD FACS lysing) procedure using antibodies against CD13, CD33, CD34, CD45, CD117, HLA-DR, CD14 and cytoplasmic MPO. Then samples were run on FACSCanto II cytometers with FACSDiva software (BD Biosciences) and data were acquired on at least 1×10^4 of total when specimen quality allowed. A blast gate was defined, encompassing CD45 dim blast population, on which Myeloid and immature marker expression was analysed. Further, blasts were categorised by their phenotypic expression in to Multipotent progenitor (MPP), Common Myeloid Progenitor (CMP), Granulocytic Myeloid Progenitor (GMP), Granulocyte/Monocyte Progenitor (GP/MP).

2.3 Molecular and Cytogenetic Analysis

Molecular analysis for FLT3-ITD/FLT3D835 was done as per standard protocol with PCR in 91 patients, for that genomic DNA was extracted from the bone marrow or peripheral blood sample using a Qiagen DNA extraction kit (Qiagen) and extended mutation analysis was done in 22 patients on the iron torrent oncomine Myeloid assay GX on fully automated Genexus NGS system (Thermoscientific) at Molecular diagnostic lab of Institute. This panel identify 45 DNA genes and 34 RNA fusion driver genes. Including FLT3-ITD, CEBPA, JAK2, CALR, TP53, NPM1, IDH1/2, and PML-RARA, along with many other important biomarkers. Cytogenetics analysis was carried out by FISH

technique at Cytogenetic lab of Institute to detect the cytogenetic abnormalities like, t (15;17)], Inv (16;16)] and Deletion of 7q, deletion of 11q, t (8;21).

2.4 Statistical Analysis

Statistical analysis was carried out using SPSS statistical software version 20 (SPSS Inc, USA). Pearson's Chi-square test with Pearson's correlation coefficient (r) was used to assess correlation and significance between two parameters. Disease-free survival was measured from the date of achieving complete remission until the date of relapse. P values ≤ 0.05 were considered to be statistically significant.

3. RESULTS

3.1 Stages of Leukemia Differentiation Arrest in the Study Cohort

To define leukemic stage arrest we used expression of routinely used key myeloid markers MPO, CD13, CD33, CD117 along with immature marker CD34 and HLADR. Hematopoietic Stem cell (HSC) and Multipotent Progenitor (MPP) were characterized by CD34 and CD117 expression and absence of MPO. Expression of CD13 and CD33 started at the MPP stage till full maturation. MPO expression was started Common myeloid precursor (CMP) stage and Granulocyte/Monocyte precursor (GMP) had the highest MPO expression (>70%). Expression of HLA-DR was useful to distinguish Monocyte progenitor (MP, HLA-DR positive) from Granulocyte progenitor (GP, HLA-DR negative).

These six markers (CD34, CD117, CD13, CD33, MPO, and HLA-DR) used in routine AML diagnosis are differentially expressed in six stages of normal myelopoiesis. Based on these markers, all AML patients can be categorized in to their leukemia differentiation arrest. Thus, all 220 AML patients were divided into Group 1 Hematopoietic Stem Cells (HSC): CD34⁺, CD117^{-/+}, CD13⁻, CD33⁻, MPO⁻, HLA-DR⁺. Group 2 Multipotent Progenitors (MPP): CD34⁺, CD117⁺, CD13⁺, CD33⁺, MPO⁻, HLA-DR⁺. Group 3 Common Myeloid Progenitors (CMP): CD34⁺, CD117⁺, CD13⁺, CD33⁺, MPO⁺ (10-70%), HLA-DR⁺. Group 4 Granulocyte/Monocyte Progenitors (GMP): CD34⁺, CD117⁺, CD13⁺, CD33⁺, MPO⁺ (>70%), HLA-DR⁺. Group 5 Granulocyte Progenitors/Monocyte Progenitors (GP/MP): CD34⁻, CD117^{+/-}, CD13⁺, CD33⁺, MPO^{+/-}, HLA-DR^{+/-} [11].

Immunophenotypic group according to their phenotype

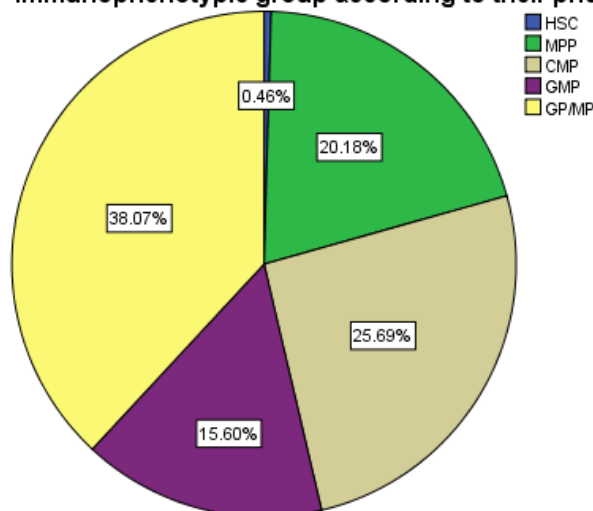


Fig. 1. AML subgroups based on their leukemic arrest using Immunophenotypic marker expression

Table 1. The distribution of various clinical and pathological parameters among different SLA groups (MPP, CMP, GMP, GP/MP) in the study cohort

Parameters	N (%)	Immunophenotypic marker expression				P
		MPP	CMP	GMP	GP/MP	
Clinical parameters						
Age	220 (100%)	47 (21%)	57 (26%)	35 (16%)	81 (37%)	
Pediatric (< 14 years)	37 (17%)	9 (24%)	12 (31%)	2 (5%)	14 (38%)	0.26
Adult (> 14 years)	183 (83%)	38 (21%)	45 (25%)	33 (18%)	67 (37%)	
Gender	220 (100%)	47 (21%)	57 (26%)	35 (16%)	81 (37%)	0.01
Male	118 (54%)	33 (28%)	34 (21%)	14 (12%)	37 (31%)	
Female	102 (46%)	14 (14%)	23 (23%)	21 (21%)	44 (43%)	
Pathological parameters						
WBC count (cells/μl)	220 (100%)	47 (21%)	57 (26%)	35 (16%)	81 (37%)	0.05
< 30150/ μ l	110 (50%)	27 (25%)	27 (25%)	23 (21%)	33 (30%)	
> 30150/ μ l	110 (50%)	20 (18%)	30 (27%)	12 (11%)	48 (44%)	
Blasts (%)	220 (100%)	47 (21%)	57 (26%)	35 (16%)	81 (37%)	0.04
< 77	112 (51%)	25 (22%)	37 (33%)	17 (15%)	33 (30%)	
> 77	108 (49%)	22 (20%)	20 (19%)	18 (17%)	48 (44%)	
FAB subtypes	220 (100%)	47 (21%)	57 (26%)	35 (16%)	81 (37%)	
M0	3 (1%)	2 (67%)	0 (0%)	0 (0%)	1 (33%)	
M1	53 (24%)	12 (23%)	12 (23%)	13 (24%)	16 (30%)	
M2	96 (44%)	23 (24%)	32 (33%)	17 (18%)	24 (25%)	0.001
M3	24 (11%)	0 (0%)	2 (8%)	2 (8%)	20 (83%)	
M4	31 (14%)	7 (23%)	9 (29%)	2 (6%)	13 (42%)	
M5	10 (5%)	2 (20%)	2 (20%)	1 (10%)	5 (50%)	
M7	3 (1%)	1 (33%)	0 (0%)	0 (0%)	2 (67%)	
Aberrant Lymphoid marker expression						
CD7	220 (100%)	47 (21%)	57 (26%)	35 (16%)	81 (37%)	0.003
Negative	60 (27%)	9 (15%)	14 (23%)	4 (7%)	33 (55%)	
Positive	160 (73%)	38 (24%)	43 (27%)	31 (19%)	48 (30%)	

Table 2. The correlation of mutational status with different SLA groups (MPP, CMP, GMP, GP/MP) in the study cohort

Parameters	N (%)	Immunophenotypic marker expression				P
		MPP	CMP	GMP	GP/MP	
Molecular mutation						
FLT-ITD	91 (100%)	19 (21%)	32 (35%)	16 (18%)	24 (26%)	0.65
Negative	76 (84%)	15 (20%)	26 (34%)	15 (20%)	20 (26%)	
Positive	15 (16%)	4 (27%)	6 (40%)	1 (7%)	4 (27%)	
FLT-D835	85 (100%)	18 (22%)	30 (35%)	15 (18%)	22 (26%)	0.46
Negative	80 (94%)	16 (20%)	29 (36%)	15 (19%)	20 (25%)	
Positive	5 (6%)	2 (40%)	1 (20%)	0 (0%)	2 (40%)	
NPM1	22 (100%)	0 (0%)	1 (25%)	0 (0%)	3 (75%)	0.10
Negative	18 (81%)	2 (12%)	10 (52%)	2 (13%)	4 (23%)	
Positive	4 (18%)	0 (0%)	0 (00%)	0 (00%)	4 (100%)	
CEBPA	20 (100%)	0 (0%)	0 (0%)	2 (50%)	2 (50%)	0.01
Negative	16(80%)	2 (12%)	9 (57)	0 (0%)	5 (31%)	
Positive	4 (20%)	0 (0%)	0 (0%)	2 (50%)	2 (50%)	
C-KIT	32 (100%)	4 (13%)	17 (53%)	5 (16%)	6 (18%)	
Negative	29 (91%)	4 (14%)	15 (52%)	5 (17%)	5 (17%)	
Positive	3 (9%)	0 (0%)	2 (67%)	0 (0%)	1 (33%)	0.70
RUNX-1	30 (100%)	5 (17%)	10 (33%)	3 (10%)	12 (40%)	0.86
Negative	17 (57%)	2 (12%)	6 (35%)	2 (11%)	7 (41%)	
Positive	13 (43%)	3 (23%)	2 (30%)	1 (08%)	5 (38%)	

In the studied cohort, the most abundant Immunophenotypic group was Group 5 (GP/MP) with 81 cases (37%), followed by Group 3 (CMP) with 57 cases (26%), Group 2 (MPP) with 47 cases (21%), and Group 4 (GMP) with 35 cases (16%). Group 1 (HSC) had 0 cases.

3.2 Correlation of SLA Subgroups with Clinicopathological Parameters

In relation with Clinical parameters, male patients had maturation arrest at initial stage of differentiation MPP (28%), CMP (21%) as compared to females, showing maturation arrest at later stage GP/MP (44%) (P=0.013). No such correlation was observed with age.

In relation to pathological parameters, high WBC was observed in advance stage of maturation GP/MP (44%) as compare to earlier stage MPP/CMP (25%) (P=0.05). Similarly, high blast count was observed in advance stage of maturation GP/MP (44%) as compare to earlier stage MPP/CMP (22%) (P=0.04).

In relation with FAB subtype, significant correlation of FAB subclass of AML was observed with Immunophenotypic maturation arrest. 67% of AML-M0 patients exhibited MPP phenotype, 30% of AMLM1 with GP/MP phenotype, 33% of AML-M2 with CMP, 83% AML M3 patients with GP/MP and 42% AML-M4 and 50% AML-M5 patients with GP/MP phenotype

(P=0.01). Moreover, initial stage of maturation was MPP (24%) was associated with expression of aberrant CD7 positive AML as compared to CD7 negative AML (15%).

3.3 Correlation of SLA Subgroups with Molecular Parameters

Out of 220 patients, FLT3-ITD/FLT3-D835 mutation was carried out in 91 patients and extended NGS panel was run in 22 patients, of which some gene mutations (ABL1, ASXL1, BCOR, CREBBP, EZH2, KMT2A, MECM, MRTFA, NUP214, PDGFRB, SF3B1, SRSF2, U2AF1, ZRSR2) not detected in any analyzed patients. While STAG2, TP53, BCOR was detected in only single patient., therefore these mutations were not included in further analysis.

In relation with FLT-ITD maturation arrest at initial stage of differentiation MPP (27%), CMP (40%) as compared to FLT3-ITD negative patients, Also, patients with FLT3-D835 mutation showing maturation arrest at MPP (40%) and CMP (20%) stage. Similar results were observed with patients with C-kit mutation showing maturation arrest at early stage CMP (67%). In contrast, majority of patients with NPM mutation (75%) showed differentiation arrest at progenitor stage (GP/MP) stage as compared to NPM negative patients (1%).

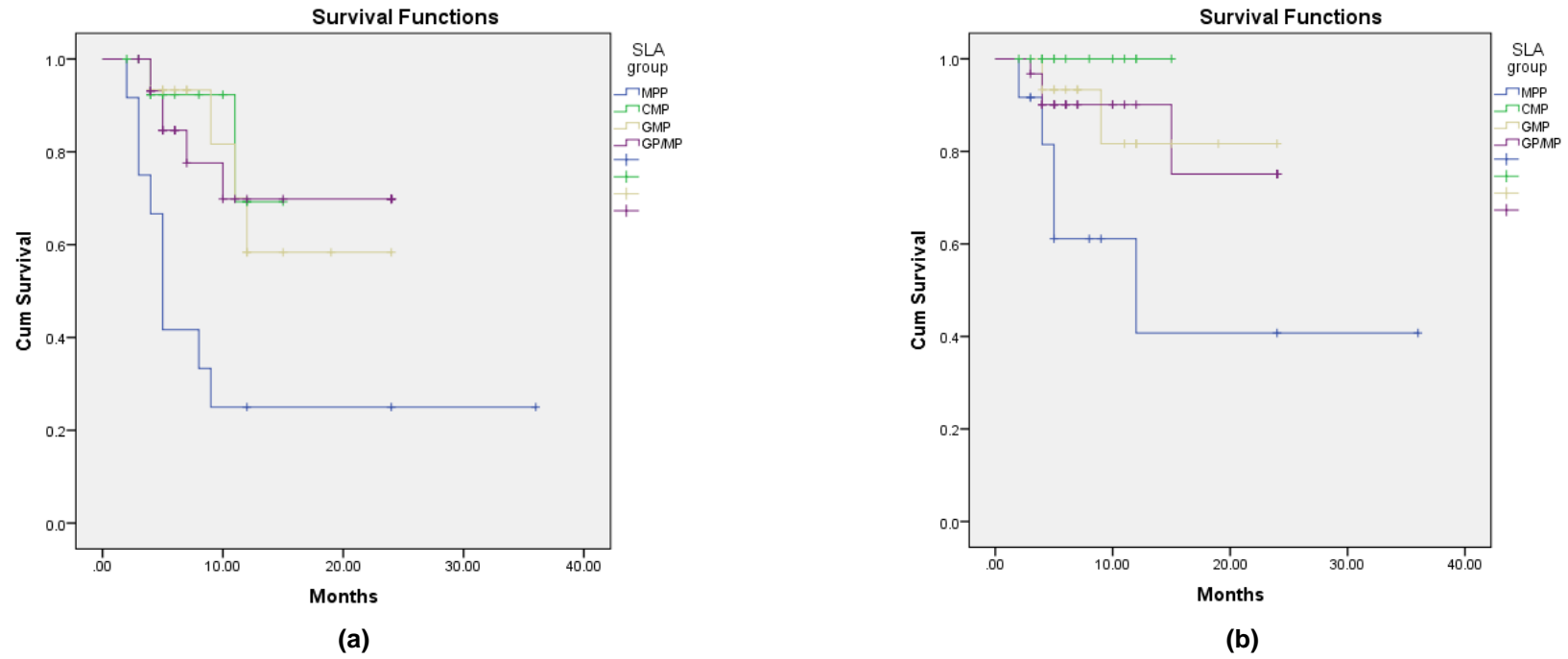


Fig. 2(a). Correlation of SLA phenotype with standard Ara(C) treatment (b) Correlation of SLA phenotype with overall survival of AML patients

Table 3. The correlation of cytogenetics status with different SLA groups (MPP, CMP, GMP, GP/MP) in the study cohort

Parameters	N (%)	Immunophenotypic marker expression				P
		MPP	CMP	GMP	GP/MP	
t(8;21)	141 (100%)	32 (23%)	44 (31%)	26 (18%)	39 (27%)	0.37
Negative	126 (89%)	26 (21%)	41 (33%)	24 (19%)	35 (28%)	
Positive	15 (11%)	6 (40%)	3 (20%)	2 (13%)	4 (27%)	
dele (7q)	7 (100%)	6 (86%)	0 (0%)	0 (0%)	1 (14%)	0.49
Negative	5 (71%)	4 (80%)	0 (0%)	0 (0%)	1 (20%)	
Positive	2 (29%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)	
Inv (16)	137 (100%)	31 (23%)	41 (30%)	27 (20%)	38 (27%)	0.31
Negative	134 (98%)	31 (23%)	41 (31%)	26 (19%)	36 (27%)	
Positive	3 (2%)	0 (0%)	0 (0%)	1 (33%)	2 (67%)	
t(15;17)	58 (100%)	10 (17%)	11(18%)	10 (17%)	27 (47%)	0.001
Negative	34 (59%)	10 (29%)	8 (23.5%)	8 (23.5%)	8 (23.5%)	
Positive	24 (41%)	0 (0%)	3 (13%)	2 (8%)	19 (79%)	

Table 4. Assessment of standard chemotherapy response following the completion of induction therapy in SLA group

SLA Group	N (%)	Persistent Leukemic activity	Post induction Remission	0.003
MPP	12 (100%)	9 (75%)	3(25%)	
CMP	15 (100%)	2 (13%)	13(87%)	
GMP	15 (100%)	4 (27%)	11(73%)	
GP/MP	31(100%)	6 (19%)	25(81%)	

3.4 Correlation of SLA subgroups with Cytogenetics

In relation with cytogenetics, patients with t (15;17) showed maturation arrest at later stage of differentiation GP/MP (79%), as compared to t(15;17) negative patients (P=0.001), Also, patients with inversion 16 showing maturation arrest at GP/MP (67%) stage. In contrast t(8;21) AML1-ETO was observed at arrest at early stage MPP (40%) and CMP (20%). Similarly, 7q deletion was observed at MPP stage (100%). FISH11q was also performed in 28 cases but all the cases were negative for abnormality of 11q chromosome.

3.5 Correlation with Treatment Outcome and Disease Status

Out of 218 patients, detail treatment history was available in 175 patients, of which 73 patients has received standard chemotherapy with Ara-C and had follow-up up to 24 months. Therefore, disease status and early treatment response of Ara-C (post induction remission) was correlated with SLA groups in these 73 patients. Out of 73 patients, 75% patients with MPP, 13% with CMP,

27% with GMP and 19% with GP/MP phenotype had persistent leukemic activity (P=0.003).

Similarly, patients with MPP phenotype showed significantly reduced overall survival as compared with other groups (P=0.026).

4. DISCUSSION

Hematopoiesis is the process through which blood cells develop progressively from immature precursors to more specific mature blood cells. Initially, hematopoietic stem cells have the potential to develop into a diverse range of blood cell types. However, as these cells mature, their differentiation potential becomes more restricted, and each stage in this hierarchical process generates increasingly specialized cells. In a similar hierarchical manner, leukemic blasts in acute myeloid leukemia (AML) also follow a structured development path. It begins with leukemia stem cells (LSCs) that have the capacity for self-renewal and differentiation into precursor cells. When mutations cause a disruption at any stage of this maturation process, it leads to an abnormal proliferation of leukemic blasts [12-14].

In this study, we aimed to understand the pathogenesis of acute myeloid leukemia (AML) within an immunophenotypic context for stage of leukemic arrest (SLA) during maturation process, focusing on five distinct subgroups of AML (HSC, MPP, CMP, GMP, GP/MP) based on routinely used surface markers (MPO, CD34, CD13, CD33, CD117, HLA-DR, CD14, CD15). We correlated these subgroups with clinicohematological parameters, mutational status, and therapy response.

In our study, none of the patients exhibited the HSC phenotype. The distribution of the other phenotypes showed 21% with MPP, 26% with CMP, 21% with GMP, and 37% with GP/MP. In relation to clinicohematological parameters, male patients displayed maturation arrest at an earlier stage of differentiation (MPP/CMP), whereas female patients showed maturation arrest at a later stage (GP/MP). Patients with high white blood cell (WBC) and blast counts exhibited maturation arrest at the later stage (GP/MP). Regarding the FAB classification, these subgroups correlate well with the FAB subtypes, 83% AML-M3, 42% of AML-M4, and 50% of AML-M5 are associated with the GP/MP phenotype, while 67% of AML-M0 and 23% of AML-M1 are associated with the MPP phenotype. However, no such correlation was observed with AML-M2. Additionally, maturation arrest at the MPP level was associated with aberrant CD7 expression.

Gene mutations play a significant role in hematopoietic differentiation and are linked to specific maturation stages. In this study, mutations in FLT3-ITD and FLT3 TKD are associated with early differentiation stages, including MPPs (27%) and CMPs (40%). C-kit mutations are also connected to early maturation arrest, primarily at the CMP stage (67%). In contrast, NPM1 mutations (75%) and CEBPA mutations (50%) are related to differentiation arrest at the progenitor stage (GP/MP). Our analysis did not find a correlation between RUNX1 mutations and SLA stages, which differs from the findings of François Vergez et al. They observed a significant association between RUNX1 mutations and the MPP phenotype. However, like our study, their research also found that NPM1 and CEBPA mutations are associated with the progenitor stage (GMP, GP/MP level). However, this results should be confirming on larger sample size. In relation to cytogenetic analysis, the t(15;17) translocation and inversion 16 are

associated with the GP/MP phenotype, whereas the t(8;21) translocation is correlated with early maturation arrest at the MPP and CMP levels.

To examine the correlation between SLA groups and treatment response, we analyzed disease status in relation to SLA in 73 patients who were followed for 24 months and treated with standard chemotherapy along with Ara-C. Among these patients, 75% with the MPP phenotype, 13% with CMP, 27% with GMP, and 19% with GP/MP showed persistent leukemic activity. This suggests that the MPP group is the most resistant to chemotherapy, followed by CMP, GMP, and GP/MP. Additionally, patients with the MPP phenotype had significantly lower overall survival compared to other groups. Both in vitro chemosensitivity studies and clinical data indicate that the SLA phenotype can predict responses to the main AML therapies. Specifically, genetic subgroups such as CEBPA, and NPM1 mutations, which fall under GMP-L/GPL/MP-L, tend to be more chemosensitive and benefit more from intensive chemotherapy compared to HSCL/MPP-L/CMP-L. Exploring the impact of new therapeutic combinations, such as azacitidine and venetoclax, [15-17] along with mutational status in this context would be highly interesting [18,19].

5. CONCLUSION

AML Immunophenotyping can identify a new SLA phenotype that effectively correlates with leukemic cell behavior and helps predict major genetic subgroups at diagnosis, along with their response to standard intensive chemotherapy. This could help in clinical management strategies. However, the molecular complexity of AML indicates the possible existence of sub clones at diagnosis [20-23]. Therefore, further research using additional genetic and immunophenotypic markers is needed to fully understand the relationship between SLA and AML.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during editing of this manuscript. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology.

Details of the AI usage are given below:

1. Chat GPT

CONSENT

General consent form was taken at the time of First Hospital visit for Outpatient Department, used in the present study.

ETHICAL APPROVAL

This study was approved by Internal Review committee and Ethical committee of Institute,

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Nalage DN, Kudnar PS, Langhe R, et al. Leukemia in India: Insights Into Incidence, Prevalence, Mortality, and Disability-Adjusted Life Years. *Cureus*. June 17, 2024;16(6):e62557. DOI: 10.7759/cureus.62557
2. Ghosh S, Shinde SC, Kumaran GS, Sapre RS, Dhond SR, Badrinath Y, et al. Haematologic and immunophenotypic profile of acute myeloid leukemia: An experience of Tata Memorial Hospital. *Ind J Cancer*. 2003;40(2):71-76.
3. Salem DA, El-Aziz SMA. Flowcytometric immunophenotypic profile of acute leukemia: mansoura experience. *Ind J Hematol Blood Transfus*. 2012;28(2):89-96.
4. Majeti R, Park CY, Weissman IL. Identification of a hierarchy of multipotent hematopoietic progenitors in human cord blood. *Cell Stem Cell*. 2007;1:635-45.
5. Doulatov S, Notta F, Eppert K, Nguyen LT, Ohashi PS, Dick JE. Revised map of the human progenitor hierarchy shows the origin of macrophages and dendritic cells in early lymphoid development. *Nat Immunol*. 2010;11:585-93.
6. Akashi K, Traver D, Miyamoto T, Weissman IL. A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. *Nature*. 2000;404:193-7.
7. Meyer SC, Levine RL. Translational implications of somatic genomics in acute myeloid leukaemia. *Lancet Oncol*. 2014;15:e382-94.
8. Genovese G, Kahler AK, Handsaker RE, Lindberg J, R Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med*. 1997;3:730-7.
9. Sarry JE, Murphy K, Perry R, Sanchez PV, Secreto A, Keefer C, et al. Human acute myelogenous leukemia stem cells are rare and heterogeneous when assayed in NOD/SCID/IL2Rgammac-deficient mice. *J Clin Investig*. 2011;121:384-95.
10. Goardon N, Marchi E, Atzberger A, Quek L, Schuh A, Soneji S, et al. Coexistence of LMPP-like and GMP-like leukemia stem cells in acute myeloid leukemia. *Cancer Cell*. 2011;19:138-52.
11. Vergez F, Largeaud L, Bertoli S. et al. Phenotypically-defined stages of leukemia arrest predict main driver mutations subgroups, and outcome in acute myeloid leukemia. *Blood Cancer J*. 2022;12:117. Available:https://doi.org/10.1038/s41408-022-00712-7
12. Bene MC, Castoldi G, Knapp W, Ludwig WD, Matutes E, Orfao A, et al. Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). *Leukemia*. 1995;9:1783-6.
13. Gorgens A, Radtke S, Mollmann M, Cross M, Durig J, Horn PA, et al. Revision of the human hematopoietic tree: Granulocyte subtypes derive from distinct hematopoietic lineages. *Cell Rep*. 2013;3:1539-52.
14. Velten L, Haas SF, Raffel S, Blaszkiewicz S, Islam S, Hennig BP, et al. Human haematopoietic stem cell lineage commitment is a continuous process. *Nat Cell Biol*. 2017;19:271-81.
15. DiNardo CD, Wei AH. How I treat acute myeloid leukemia in the era of new drugs. *Blood* 2020; 135:85-96.
16. DiNardo CD, Jonas BA, Pullarkat V. Azacitidine and venetoclax in previously untreated acute myeloid leukemia N. *Engl. J. Med*. 2020;383:617-629.
17. Pino JC, Posso C, Joshi SK, Nestor M, Moon J, Hansen JR, Hutchinson-Bunch C, Gritsenko MA, Weitz KK, Watanabe-Smith K, Long N, McDermott JE, Druker BJ, Liu T, Tyner JW, Agarwal A, Traer E, Piehowski PD, Tognon CE, Rodland KD, Gosline SJC. Mapping the proteogenomic landscape enables prediction of drug

- response in acute myeloid leukemia. Cell Rep Med. 2024 Jan 16;5(1):101359. DOI: 10.1016/j.xcrm.2023.101359. PMID: 38232702; PMCID: PMC10829797
18. Jayavelu AK, Wolf S, Buettner F, Alexe G, Häupl B, et al. The proteogenomic subtypes of acute myeloid leukemia. Cancer Cell. 2022 Mar 14;40(3):301-317.e12. DOI: 10.1016/j.ccell.2022.02.006. Epub 2022 Mar 3. PMID: 35245447
19. Bottomly D, Long N, Schultz AR, Kurtz SE, Tognon CE, Johnson K, Abel M, Agarwal A, Avaylon S, Benton E, et al. Integrative analysis of drug response and clinical outcome in acute myeloid leukemia. Cancer Cell. 2022;40:850–864.e9.
20. Bottomly D, Long N, Schultz AR, Kurtz SE, Tognon CE, et.al, Integrative analysis of drug response and clinical outcome in acute myeloid leukemia. Cancer Cell. 2022 Aug 8;40(8):850-864.e9. DOI: 10.1016/j.ccell.2022.07.002. Epub 2022 Jul 21. PMID: 35868306; PMCID: PMC9378589
21. Corces-Zimmerman MR, Hong WJ, Weissman IL, Medeiros BC, Majeti R. Preleukemic mutations in human acute myeloid leukemia affect epigenetic regulators and persist in remission. Proc Natl Acad Sci USA. 2014;111:2548–53.
22. Klco JM, Spencer DH, Miller CA, Griffith M, Lamprecht TL, O’Laughlin M, et al. Functional heterogeneity of genetically defined subclones in acute myeloid leukemia. Cancer Cell. 2014;25: 379–92.
23. Röhrs S, Scherr M, Romani J, Zaborski M, Drexler HG, Quentmeier H. CD7 in acute myeloid leukemia: correlation with loss of wild-type CEBPA, consequence of epigenetic regulation. Journal of hematology & oncology. 2010 Dec; 3:1-7.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of the publisher and/or the editor(s). This publisher and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

© Copyright (2024): Author(s). The licensee is the journal publisher. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
<https://www.sdiarticle5.com/review-history/123475>