# Clinicohematological Parameters and Assessment of Post Induction Status in Acute Myeloid Leukemia–Experience at A Tertiary Care Center

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## ABSTRACT

***Objective***: To evaluate the role of clinical, hematological and genetic parameters in acute myeloid leukemia (AML) as the predictors of response to induction chemotherapy.

***Study Design***: Cross-sectional study.

***Place and Duration of Study***: Department of Haematology, Armed Forces Institute of Pathology, from Jun to Jun 2019.

***Methodology***: All the newly diagnosed cases of de-novo AML were included in the study. Clinical, haematological and immunophenotypic parameters were noted. Cytogenetic and molecular analyses were performed. Response to first course of standard induction chemotherapy was assessed.

***Results***: A total of 58 patients were included in the study. The median age was 19 years. Thirty-one (53.4%) were adults while 27 (46.6%) were in the pediatric age group. Thirty-six (62.1%) were males while 22 (37.9%) were females. The most common clinical presentation was fever. The most common French-American-British classification (FAB) subtype was AML-M2. The blast percentage was 78%. Forty-six (79.3%) patients had a normal karyotype. Of the 58 patients, 38 (65.5%) achieved complete remission while 20 (34.5%) did not achieve complete remission after induction chemotherapy.

***Conclusion***: AML was seen in a younger age group in our population. There was statistically significant association of high white blood cell (WBC) count with remission status. Increasing age was associated with a poor response to induction chemotherapy, while translocation t(8;21) was associated with a good response. Assessment of prognostic parameters is vital in the initial diagnostic workup of AML patients to predict response to induction chemotherapy.

**Keywords**: Acute myeloid leukemia (AML), Complete remission (CR), Cytogenetics, Prognostic markers.

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## INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous group of malignant hematopoietic disorders resulting from clonal proliferation of abnormal myeloid blasts.[1] This results in loss of normal hematopoiesis, as the malignant blasts accumulate in the bone marrow, thus causing the symptoms of bone marrow failure i.e. anemia, infection and bleeding. Some patients may have extramedullary blast proliferation.[2]

Globally, the incidence of AML is around 2.5-3 cases per 100,000 population per year. It can occur at all ages, but the median age at diagnosis reported in the western population is 69 years.[3] The incidence increases with age and it has a slight male preponderance.[4] It accounts for 15-20% of childhood acute leukemias and 80% of adult acute leukemias.[5]

AML has been classified traditionally using the FAB (French-American-British) classification, which is based on morphology and cytochemistry, but immunophenotyping may be required to make a final diagnosis in some cases. FAB classification divides AML into 8 subtypes, from M0 to M7.[6] However; World Health Organization (WHO) has classified these disorders based on cytogenetic and molecular markers. The WHO criteria define AML by the presence of 20% or more myeloid blasts in the peripheral blood or bone marrow.[3,7] A lower blast percentage is acceptable if specific genetic changes are present i.e. t (8;21), inv (16) or t (16;16) and t (15;17). Immunophenotyping by flow cytometry, where available, is helpful in diagnosing and subtyping AML. Karyotype analysis and molecular testing for the gene rearrangements and mutations common in AML should also be done where available, as there are 11 AML subtypes which are defined by the presence of certain recurrent cytogenetic or molecular aberrations in the 2016-updated WHO classification.[8] These and other genetic abnormalities, not included in the classification, also have established prognostic implications and have been used to classify patients into risk stratified groups.[9]

The treatment response and outcome in AML is diverse and depends on various prognostic factors. Acute myeloid leukemias are seen in both Pakistani adults as well as children. Armed Forces Institute of

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Pathology is a tertiary care referral center providing morphological diagnosis as well as advanced ancillary facilities like immunophenotyping, cytogenetics, FISH analysis and testing for molecular markers including those for recurrent genetic abnormalities. The current study was aimed at analyzing the clinical, hematological, immunophenotypic and genetic features of these AML patients and the assessment of their impact on post-induction remission status.

## METHODOLOGY

This cross-sectional study was conducted at the Haematology Department, Armed Forces Institute of Pathology, Rawalpindi, from Jun to Jun 2019.

**Inclusion Criteria**: Newly diagnosed cases of AML of both genders and all age groups were included.

**Exclusion Criteria**: Previously diagnosed or relapsed cases of AML, AML transformed from other hematological disorders and patients with history of chemotherapy or radiotherapy were excluded from the study. Patients diagnosed as having acute promyelocytic leukemia (AML M3) were also excluded from the study as these patients receive a different induction protocol.

Study was conducted after the approval of the Institutional Review Board (IRB no. 18/860) and written informed consent was taken from the patients. Sample size was calculated using WHO sample size calculator. Peripheral blood and bone marrow samples were collected. AML was diagnosed on the basis of WHO criteria,[10] with the morphological assessment of the peripheral blood and bone marrow (aspirates/biopsies). All the cases were also assigned with the FAB subtypes.

A detailed history was taken and physical examination was performed. Age, gender, presenting complaints and physical findings were noted on a proforma. Complete blood counts were performed on Sysmex automated hematology analyzer XN-3000 and the hematological parameters were recorded. Bone marrow examination (Aspiration and Trephine biopsy) was performed. Peripheral blood and bone marrow smears were stained with Leishman and Giemsa stain. Bone marrow blast morphology and percentage were noted.

Immunophenotyping was performed in all the patients at the time of diagnosis. 100uL of (EDTA anticoagulated) bone marrow/peripheral blood sample was used. Two-colour flow cytometry was performed on FACS Flow Cytometer (Becton-Dickinson Biosciences USA) using monoclonal antibodies provided by Becton-Dickinson Biosciences USA.

Cytogenetic analysis was performed on metaphase cells by the conventional Giemsa-trypsin banding technique using bone marrow/peripheral blood samples. Patients were then classified according to their karyotype. RNA was extracted for RT-PCR of acute myeloid gene panel including RUNX1-RUNX1T1, CBFB-MYH11 and DEK-NUP214.

All the patients were treated with standard induction chemotherapy i.e Daunorubicin in a dose of 45-50 mg/m$^2$ for 3 days and Cytarabine (Ara-C) in a dose of 100 mg/m$^2$ for 7-10 days (D3A7 protocol for adult patients and D3A10 protocol for paediatric patients). Blood counts and bone marrow examination were performed after the first induction cycle to assess response. Complete remission (CR) was defined according to the 2017 ELN guidelines: 9 ANC >1.0 x 10$^9$/L, platelet count >100 x 10$^9$/L, blasts in bone marrow <5%, absence of blasts in peripheral blood and blasts with Auer rods, absence of extramedullary disease.

Data was analyzed by using Statistical Package for Social Sciences (SPSS) version 24. Comparison of clinicohaematological, cytogenetic and molecular characteristics of patients with remission status was done by using chi square test. The *p*-value of ≤0.05 was considered statistically significant.

## RESULTS

A total of 58 newly diagnosed AML patients were included in the study. The median age of our study group was 19 years, with an age range of 9 months-68 years. Thirty-one (53.4%) patients were adults while 27 (46.6%) were in the paediatric age group. Of these AML patients, 36 (62.1%) were males while 22 (37.9%) were females. On detailed history and physical examination, the most common clinical presentation was fever in 56 (96.6%) patients followed by pallor in 44 (75.9%) and easy fatigability/lethargy in 39 (67.2%) patients as shown in the Figure.

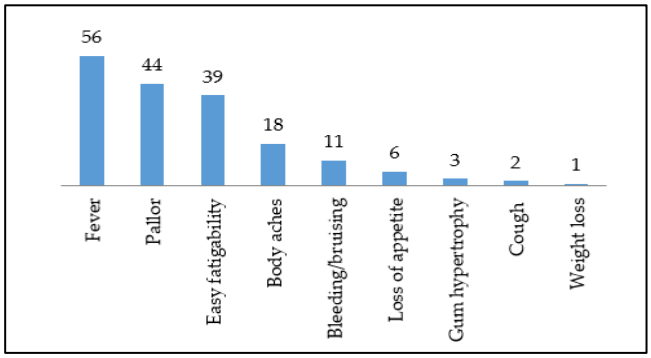


**Figure: Clinical features of the study population.**

The median hemoglobin concentration was 8.2 g/dL, median WBC count was 26 x $10^9$/L, median platelet count was 76 x $10^9$/L and the blast percentage was 78%. According to the FAB classification, 4 (6.9%) were classified as AML-M0, 6 (10.3%) as AML-M1, 28 (48.3%) as AML-M2, 10 (17.2%) as AML-M4, 4 (6.9%) as AML-M5 and 6 (10.3%) as AML-M6, while morphologically no patient was classified as AML-M7.

On immunophenotyping by flow cytometry, the myeloid markers CD13 and CD33 were positive in 54 (93.1%) patients. Fifty (86.2%) patients were positive for MPO while 8 (13.8%) patients were negative for MPO. Aberrant expression of CD7 was seen in 18 (31%) patients. Cytogenetic analysis was performed for each of these patients. Conventional cytogenetics revealed that 46 (79.3%) patients had a normal karyotype while 11 (19%) had t (8;21) and 1 (1.7%) had a complex karyotype. Molecular analysis by real-time PCR detected RUNX1-RUNX1T1 fusion gene in 11 (19%) patients, CBFB-MYH11 fusion gene in 2 (3.4%) patients and DEK-NUP214 rearrangement in 1 (1.7%) patients.

Each patient was assessed for remission status after receiving first course of standard induction chemotherapy. Thirty-eight (65.5%) of the patients achieved complete remission while 20 (34.5%) did not. We then compared the clinico-haematological, cytogenetic and molecular characteristics of both groups as shown in the Table.

## DISCUSSION

Over the years the heterogeneity of AML with respect to its clinical, hematological, and genetic factors has been well established.[10] The treatment response and the overall survival in AML depend on prognostic factors which can be divided into patient-associated factors and disease-associated factors.[11,12] These factors help in devising the treatment plan, including the decision of allogenic transplantation and targeted therapies.[13] Among the patient-related parameters, age is the most significant, and among the disease-related parameters, genomic lesions are the strongest predictors of disease behavior.[12,14] Development in understanding of molecular pathways in recent years has not only enhanced our knowledge regarding disease pathophysiology but has also led to the identification of a number of factors important in determining prognosis.

Considering the diversity of disease presentation and disease course as well as the racial, ethnic and geographic differences, we studied these clinico-haematologic parameters, immunophenotypic, cytogenetic and molecular factors to identify disease charac-teristics in our population.

**Table: Comparison of clinicohaematological, cytogenetic and molecular characteristics of patients in remission and those who failed to achieve remission.**

| Parameters | In Remission (n=38) | | Not in Remission (n=20) | | *p*-value |
|---|---|---|---|---|---|
| | n | % | n | % | |
| **Age (years)** | | | | | |
| <12 years | 20 | 52.6 | 7 | 35 | 0.20 |
| 13-50 years | 13 | 34.2 | 7 | 35 | |
| >50 years | 5 | 13.1 | 6 | 30 | |
| **Gender** | | | | | |
| Male | 26 | 68.4 | 10 | 50 | 0.10 |
| Female | 12 | 31.6 | 10 | 50 | |
| **Blood Counts** | | | | | |
| WBC | | | | | 0.01 |
| <10x$10^9$/l | 7 | 18.4 | 2 | 10 | |
| 10-50x$10^9$/l | 26 | 68.4 | 8 | 40 | |
| > 50x$10^9$/l | 5 | 13.1 | 10 | 50 | |
| **Haemoglobin** | | | | | |
| <10g/dl | 32 | 84.2 | 19 | 95 | 0.20 |
| >10g/dl | 6 | 15.8 | 1 | 5 | |
| **Platelets** | | | | | |
| <100x$10^9$/l | 34 | 89.5 | 20 | 100 | 0.10 |
| >100x$10^9$/l | 4 | 10.5 | - | - | |
| **FAB Type** | | | | | |
| M0 | 1 | 2.6 | 3 | 15 | 0.30 |
| M1 | 4 | 10.5 | 2 | 10 | |
| M2 | 21 | 55.2 | 7 | 35 | |
| M4 | 7 | 18.4 | 3 | 15 | |
| M5 | 2 | 5.26 | 2 | 10 | |
| M6 | 3 | 7.9 | 3 | 15 | |
| M7 | - | - | - | - | |
| **Cytogenetics** | | | | | |
| Normal karyotype | 29 | 76.3 | 17 | 85.0 | 0.1 |
| t (8;21) | 9 | 23.7 | 2 | 10.0 | |
| Complex karyotype | - | - | 1 | 5.0 | |
| **Molecular Markers** | | | | | |
| RUNX1-RUNX1T1 | 9 | 23.7 | 2 | 10.0 | 0.1 |
| CBFB-MYH11 | 2 | 5.26 | - | - | |
| DEK-NUP214 | - | - | 1 | 5.0 | |

The median age of our patients was 19 years, with an age range of 9 months-68 years. Jahic *et al*,[15] has reported a much higher age of 53 years in Bosnian population. Zheng *et al*,[16] has a median age of 23.7 years. In our study group, 36 (62.1%) patients were males while 22 (37.9%) were females with a male to female ratio of 1.6:1. Mahmood *et al*,[17] and Mohammed *et al*,[18] have observed similar male predominance among AML patients. However, a study conducted in Emam Reza

Hospital in Iran,[19] has reported 40.6% males and 59.4% females.

Byrd *et al*,[20] in his study on AML patients has reported median hemoglobin of 9.2 g/dL, median WBC count of 18.2 x $10^9$/L and median platelet count of 57 x $10^9$/L. These results were comparable to the blood counts in our patients. The bone marrow blast percentage in our study was 78% while Mohammed *et al*,[18] has reported blast percentage of 66%.

The most common FAB type was AML-M2 seen in 28 (48.3%) patients, followed by AML-M4 in 10 (17.2%) patients, while morphologically no patient was classified as AML-M7. Wakui *et al*,[21] observed similar findings in Japanese AML patients, with AML-M2 being the most common type followed by AML-M4, while AML-M7 was seen in 5 (0.8%) patients.

In our study, out the 58 patients, 38 (65.5%) achieved complete remission while 20 (34.5%) did not achieve complete remission after induction chemotherapy. It was noted that increasing age was associated with lower remission rates. This was consistent with the study conducted by Appelbaum on the American patients.[22] There was no statistically significant association of gender with remission status. There was statistically significant association of high WBC count (>50 x $10^9$) with remission status (*p*-value 0.01), with 66.7% of these patients failing to achieve CR after induction chemotherapy. This was in accordance with a study conducted in Brazil.[23] Hyperleukocytosis (initial WBC >50 x $10^9$) has been reported to be an independent adverse prognostic factor in AML.[24] It was observed that majority of the patients presenting at our institute had low haemoglobin levels (<10 g/dL) and low platelet count (<100 x 109).

Response to induction treatment is a predictor of risk of relapse and overall survival.[25] Good response was seen in patients with AML-M2 followed by patients in the FAB group AML-M4, while poor response was seen in the FAB type M0.

Patients with t (8;21) had higher remission rates, while one patient with complex karyotype failed to achieve CR. Patients having RUNX1-RUNX1T1 and CBFB-MYH11 rearrangements showed good response to induction therapy, while those harboring DEK-NUP214 fusion gene failed to achieve CR.

Importance of the prognostic markers of AML cannot be overlooked. Assessment of these parameters is vital in the initial diagnostic workup of AML patients. Clinical presentation and hematological findings are helpful in predicting disease course and tailoring the management plan. Cytogenetic and molecular markers are essential for not only the diagnosis and prognosis but also have a role in the monitoring of minimal residual disease. Further studies with larger sample size and longer follow up period are required to establish the effect of these parameters on the overall survival in our population.

## CONCLUSION

AML was seen in a younger age group in our population. There was statistically significant association of high white blood cell (WBC) count with remission status. Increasing age was associated with a poor response to induction chemotherapy, while translocation t(8;21) was associated with a good response. Assessment of prognostic parameters is vital in the initial diagnostic workup of AML patients to predict response to induction chemotherapy.

**Conflict of Interest:** None.

**Disclosure**: The abstract has been presented as an E-poster in Haemcon 2020, Lahore, Pakistan.

**Authors' Contribution**

SAZ: Direct contribution, AM:, RM:, AK:, SA:, AL: Intellectual contribution.

## REFERENCES

1. Bain BJ, Clark DM, Wilkins BS. Acute myeloid leukemia. In: Bone marrow pathology. 5th edition, Wiley-Blackwell; 2019, Available at: https://www.wiley.com/en-us/Bone+Marrow+Pathology,+5th+Edition-p-9781119398127
2. Bewersdorf JP, Zeidan AM. Hyperleukocytosis and leukostasis in acute myeloid leukemia: can a better understanding of the underlying molecular pathophysiology lead to novel treatments? Cells 2020; 9(10): 2310.
3. Arber DA, Orazi A, Hasserjian RP, Brunning RD, Le Beau MM, Porwit A. Introduction and overview of the classification of myeloid neoplasms. WHO classification of tumours of haematopoietic and lymphoid tissues. Revised 4th ed, Lyon, IARC; 2017, https://publications.iarc.fr/Book-And-Report-Series/Who-Classification-Of-Tumours/WHO-Classification-Of-Tumours-Of-Haematopoietic -And-Lymphoid-Tissues-2017
4. Juliusson G, Antunovic P, Derolf Å, Lehmann S, Möllgård L, Stockelberg D, et al. Age and acute myeloid leukemia: real world data on decision to treat and outcomes from the Swedish Acute Leukemia Registry. Blood 2009; 113(18): 4179-4187.
5. Redaelli A, Lee JM, Stephens JM, Pashos CL. Epidemiology and clinical burden of acute myeloid leukemia. Expert Rev Anticancer Ther 2003; 3(5): 695-710.
6. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposed revised criteria for the classification of acute myeloid leukemia. a report of the French-American-British Cooperative Group. Ann Intern Med 1985; 103(4): 620-625.
7. Burnett AK, Grimwade D. Acute Myeloid Leukaemia. Book Editor (s): A Victor Hoffbrand, Douglas R Higgs, David M Keeling, Atul B Mehta. Postgraduate Haematology Hoffbrand, 7th edition; 2016, Available at: https://www.wiley.com/en-us/Postgraduate+Haematology%2C+7th+Edition-p-9781118854327
8. Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, et al. Diagnosis and management of AML in adults:

2017 ELN recommendations from an international expert panel. Blood 2017; 129(4): 424-447.
9. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med 2016; 374(23): 2209-2221.
10. Short NJ, Rytting ME, Cortes JE. Acute myeloid leukaemia. Lancet 2018; 392(10147): 593-606.
11. Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. N Engl J Med 2015; 373(12): 1136-1152.
12. Liersch R, Müller-Tidow C, Berdel WE, Krug U. Prognostic factors for acute myeloid leukaemia in adults-biological significance and clinical use. Br J Haematol 2014; 165(1): 17-38.
13. Grimwade D, Hills RK. Independent prognostic factors for AML outcome. Hematology Am Soc Hematol Educ Program 2009; 2(1): 385-395.
14. Schoch C, Kern W, Schnittger SU, Buchner T, Hiddemann WO, Haferlach TO. The influence of age on prognosis of de novo acute myeloid leukemia differs according to cytogenetic subgroups. Haematol 2004; 89(9): 1082-1090.
15. Jahic A, Iljazovic E, Hasic S, Arnautovic AC, Sabitovic D, Mesanovic S, et al. Prognostic parameters of acute myeloid leukaemia at presentation. Med Arch 2017; 71(1): 20-24.
16. Zheng J, Wang X, Hu Y, Yang J, Liu J, He Y, et al. A correlation study of immunophenotypic, cytogenetic, and clinical features of 180 AML patients in China. Cytometry B Clin Cytom 2008; 74(1): 25-29.
17. Mahmood R, Altaf C, Malik HS. Clinico-Haematologic association and prognostic relevance of NPM1 and FLT3-ITD mutations in acute myeloid leukaemia. Pak J Med Sci 2019; 35(1): 23.
18. Muhammed NS, Shamoon RP, Pouls RK. Clinical and haematological parameters in adult AML patients: a four year experience at Nanakaly Hospital for blood diseases. Zanco J Med Sci 2012; 16(3): 199-203.
19. Allahyari A, Tajeri T, Sadeghi M. Prognostic factors and survival in acute myeloid leukemia cases: a report from the Northeast of Iran. Asian Pac J Cancer Prev 2016; 17(3): 1547-1551.
20. Byrd JC, Mrózek K, Dodge RK, Carroll AJ, Edwards CG, Arthur DC, et al; Cancer and leukemia group B (CALGB 8461). Pre-treatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). Blood 2002; 100(13): 4325-4336.
21. Wakui M, Kuriyama K, Miyazaki Y, Hata T, Taniwaki M, Ohtake S, et al. Diagnosis of acute myeloid leukemia according to the WHO classification in the Japan Adult Leukemia Study Group AML-97 protocol. Int J Hematol 2008; 87(2): 144-151.
22. Appelbaum FR, Gundacker H, Head DR, Slovak ML, Willman CL, Godwin JE, et al. Age and acute myeloid leukemia. Blood 2006; 107(9): 3481-3485.
23. Oliveira LC, Romano LG, Prado-Junior BP, Covas DT, Rego EM, De Santis GC. Outcome of acute myeloid leukemia patients with hyperleukocytosis in Brazil. Med Oncol 2010; 27(4): 1254-1259.
24. Tien FM, Hou HA, Tsai CH, Tang JL, Chen CY, Kuo YY, et al. Hyperleukocytosis is associated with distinct genetic alterations and is an independent poor-risk factor in de novo acute myeloid leukemia patients. Eur J Haematol 2018; 101(1): 86-94.
25. Øvlisen AK, Oest A, Bendtsen MD, Bæch J, Johansen P, Lyng-gaard LS, et al. Stringent or nonstringent complete remission and prognosis in acute myeloid leukemia: a Danish population-based study. Blood Adv 2018; 2(5): 559-564.