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Original Article

Clinical Profile of Acute Myeloid Leukemia in North India and Utility of Nontransplant Measures in its Management

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Abstract

Introduction: Acute myeloid leukemia (AML) is a clonal accumulation of myeloid precursors in body tissues, which ultimately leads to bone marrow failure. This is an 8-year prospective, observational study in which 254 patients were enrolled. **Aim of the Study:** To document the clinical profile of AML and differential outcome in M3 versus non-M3 phenotype and to see impact of different variables on its survival. **Methods:** Patients enrolled in the study were examined, evaluated, and given standard 3:7 induction protocol, and acute promyelocytic leukemia (APML) patients were given the ICAPL 2006 protocol. **Results:** In our study, males outnumbered females and most of our patients were in 20–60 years of age group. The better prognosis was in patients who were in the second decade of life. Total leukocyte count and platelet count had a significant impact on the survival of the a patient. Bone marrow morphology of M3 type has extremely good prognosis and was the most common FAB type seen in our study. Flow cytometric markers such as CD15, CD33, CD117, and myeloperoxidase had positivity among 90% of patients. Overall survival is around 40% in whole-study group, 87% in APML group, and 16.5% in non-M3 group. There are still unmet needs in managing the non-M3 patients in resource-constraint countries where allogenic transplant and newer drugs have the least access. For improving the outcome in M3 AML, further newer molecules such as Flt3 and PIK3 inhibitors are being used in trials. **Conclusion:** There are still unmet needs in managing the non-M3 patients in resource-constraint countries where allogenic transplant and newer drugs have the least access. For improving the outcome in M3 AML, further newer molecules such as Flt3 and PIK3 inhibitors are being used in trials.

Keywords: *Acute myeloid leukemia, acute promyelocytic leukemia, cluster differentiation, event-free survival, myelodysplastic leukemia, myeloperoxidase, overall survival, reverse-transcriptase polymerase chain reaction, total leukocyte count*

Introduction

Acute myeloid leukemia (AML) is a heterogeneous hematologic malignancy characterized by clonal expansion of myeloid blasts in the peripheral blood, bone marrow, and/or other tissues, which leads to impaired production of normal blood cells. Thus, leukemic cell infiltration in the marrow invariably leads to bone marrow failure manifesting in the form of anemia or thrombocytopenia, while absolute neutrophil count may be low or normal, depending on the total white cell count.[1]

The underlying pathophysiology in AML consists of a maturational arrest of bone marrow cells in the earliest stages of development. The mechanism of this arrest is under study, but in many cases, it involves the activation of abnormal genes through chromosomal translocations and other genetic abnormalities.[2,3] Those genetic changes can be inherited or acquired to some environmental insult. AML accounts for approximately 20% of acute leukemia in children and 80% of acute leukemia in adults. The incidence of AML progressively increases with age, and in adults over the age of 65 years, the incidence is approximately 30 times the incidence of AML in children. The highest rate of childhood AML is in Asia and the lowest in North America and India.[4]

As per the SEER database, the number of new cases of AML was 4.2 per 100,000 men and women per year. The number of deaths was 2.8 per 100,000 men and women per year. These rates are age adjusted and based on 2010–2014 cases and deaths. AML is most frequently diagnosed among people aged 65–74 years. Median age at diagnosis is 68 years.[5]

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The most common risk factor for AML is the presence of an antecedent hematologic disorder, the most common of which is myelodysplastic syndrome. Some congenital disorders that predispose patients to AML include Bloom syndrome, Down syndrome, congenital neutropenia, Fanconi anemia, and neurofibromatosis. Usually, these patients develop AML during childhood; rarely, some may present in young adulthood.

Radiation exposure, smoking, and exposure to benzene have been found to be associated with AML.

As more patients with cancer survive along their primary malignancy, the number of patients with AML increases because of exposure to chemotherapeutic agents. For example, the cumulative incidence of acute leukemia in patients with breast cancer who were treated with doxorubicin and cyclophosphamide as adjuvant therapy was 0.2%–1.0% at 5 years.[6]

Patients with previous exposure to chemotherapeutic agents can be divided into two groups: (1) those with previous exposure to alkylating agents and (2) those with exposure to topoisomerase-II inhibitors. Patients with a previous exposure to topoisomerase-II inhibitors do not have a myelodysplastic phase. Cytogenetic testing reveals a translocation that involves band 11q23. Less commonly, patients developed leukemia with other balanced translocations, such as inversion 16 or t(15;17).[7] The typical latency period between drug exposure and acute leukemia is approximately 3–5 years for alkylating agents/radiation exposure, but it is only 9–12 months for topoisomerase inhibitors.

The newer who classification is based on molecular markers and morphology [Table 1].[8] The European Leukemia Network has divided AML into three risk groups, as favorable-risk, intermediate-risk, and poor-risk groups [Table 2].[9]

Treatment is individualized as we have to distinguish M3 from non-M3 at the outset. For non-M3, induction is given which consists of mainly daunorubicin and cytarabine. Dosages of daunorubicin range from 45 to 90 mg/m^2 given day 1–day 3, while cytarabine dosage is 100–200 mg/m^2 given by 24 h infusion over 7 days. Recent studies have clearly shown dose of 60 mg/m^2 better than 45 mg/m^2, and then further, Burnett *et al*. have clearly shown 60 mg/m^2 better than 90 mg/m^2 in terms of same complete remission (CR) and lower 60-day mortality. Idarubicin can also be used in the place of daunorubicin with the same outcome. CR rates in the age group of <50 years have consistently been in the range of 60%–70% in largest cooperative group trials of infusional cytarabine and anthracyclines. Induction is followed by consolidation once remission is documented. Type of treatment mainly depends upon risk stratification. Adverse-risk patients are taken for allogenic stem cell transplant. Favorable-risk patients are to be taken for 3–4 cycles of high-dose cytarabine. Intermediate-risk patients are to be given either allogenic stem cell transplant or high-dose cytarabine depending upon the availability of donor and age of patient. Patients who fall in M3 type (acute promyelocytic leukemia [APML]) need differentiating agent as induction treatment. These agents are all-transretinoic acid (ATRA) and arsenic trioxide which are combined with conventional chemotherapy agents. Most of the studies mention CR rate of around 90%. Transplant is only used in relapsed setting.[10]

As per the SEER database, 5-year survival of AML is 26.9%.[5] Swaminathan *et al*. reported a 5-year overall survival of 30% in the Madras Metropolitan Tumor Registry.[11] Philip *et al*. reported the overall survival at 1 year of 70.4% ± 10.7%, 55.6% ± 6.8%, and 42.4% ± 15.6% in patients aged ≤15 years, 15–60 years, and ≥60 years, respectively.[12]

Aims and objectives

1. To document clinical profile of AML
2. To see differential outcome in M3 versus non-M3 phenotype

Table 1: WHO classification of acute myeloid leukemia

AML and related neoplasms
AML with recurrent genetic abnormalities
AML with t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
APL with *PML-RARA*
AML with t(9;11)(p21.3;q23.3);*MLLT3-KMT2A*
AML with t(6;9)(p23;q34.1);*DEK-NUP214*
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2*, *MECOM*
AML (megakaryoblastic) with t(1;22)(p13.3;q13.3); *RBM15-MKL1*
AML with mutated *NPM1*
AML with biallelic mutations of *CEBPA*
AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms
AML, NOS
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Pure erythroid leukemia
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma
Myeloid proliferations related to down syndrome
TAM
Myeloid leukemia associated with down syndrome

AML – Acute myeloid leukemia APL – Acute promyelocytic leukemia; NOS – Not otherwise specified; TAM – Transient abnormal myelopoiesis

Table 2: 2017 European leukemia net risk stratification of acute myeloid leukemia by genetics

Risk category*	Genetic abnormality
Favorable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
	Inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated *NPM1* without *FLT3-ITD* or with *FLT3-ITD*
	Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3-ITD*^{high}
	Wild-type *NPM1* without *FLT3-ITD* or with *FLT3-ITD*^{low} (without adverse-risk genetic lesions)
	t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*
	Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); *DEK-NUP214*
	t(v; 11q23.3); *KMT2A* rearranged
	t(9;22)(q34.1;q11.2); *BCR-ABL1*
	Inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2*, *MECOM*(*EVI1*) −5 or del (5q); −7; −17/abn(17p)
	Complex karyotype, monosomal karyotype
	Wild-type *NPM1* and *FLT3-ITD*^{high}
	Mutated *RUNX1*
	Mutated *ASXL1*
	Mutated *TP53*

3. To see impact of different variables on its survival.

Methods

This was a hospital-based, observational study, spanned over 8 years, in which the first patient was enrolled in January 2009 and the last patient was enrolled in December 2015, and complete data were taken in December 2016, given 1 year of follow-up from the last patient enrollment. Ethical clearance for this study was obtained and the study was conducted in Hemato-oncology Department of Sheri Kashmir Institute of Medical Sciences, Srinagar, India. All age groups were taken into the study and those relapsed cases were excluded from the study.

1. Detailed history from patients/attendants was taken and recorded. After informed consent for examination, methods/procedures to be used, use of data for research work and/or publications, and complete physical examination of the patient were recorded
2. Patients underwent the following investigations:
   - Complete blood count with peripheral smear, liver function tests, kidney function tests, lactate dehydrogenase level, blood sugar, uric acid, electrocardiogram, chest X-ray, urine examination, hepatitis and HIV serology, and electrolytes
   - Bone marrow aspiration and biopsy for morphology, cytochemistry, flow cytometry, and cytogenetics. For morphology of the bone marrow, Leishman stain was used. For cytochemistry, stains such as myeloperoxidase (MPO), Sudan black B, and periodic acid–Schiff were used. Cytogenetics was done by conventional karyotyping, in which at least 20 metaphases were analyzed
   - Documentation of complete hematologic remission and bone marrow remission after induction on morphology
3. Outcome of the treatment and the type of treatment received (standard treatment or supportive care)
4. Survival duration since diagnosis up to the last censored date of December 30, 2016. Patients whose outcome was unknown were not taken for survival analysis. Outcome was correlated with different prognostic variables
5. Patients were divided into M3 (promyelocyte leukemia) and non-M3 myeloid leukemia.

The data were analyzed using descriptive statistics. The patients were divided into two groups, alive and dead. Qualitative variables were compared using the Chi-square test. A univariate analysis was carried out to identify the variables with a significant association with relapse or death. A "*P*" <0.05 was considered statistically significant. Survival was measured from date of initial diagnosis of AML to date of death from any cause using the Kaplan–Meier method, which is a nonparametric (actuarial) technique for estimating time-related events (the survivorship function).

Results and Observations

This was a hospital-based, observational study, spanned over 8 years, in which the first patient was enrolled in January 2009 and the last patient was enrolled in December 2015, and complete data were taken in December 2016, given 1 year of follow-up from the last patient enrollment. A total of 254 patients were enrolled. Average number of cases per year was 36 [Figure 1].

Males were 142 and females were 112; ratio was 1.2:1. With respect to FAB type, i.e. M3 and non-M3, there was equal number of male and female. Patients were divided into four groups based on their age groups, viz., <10 years, 11–20 years, 21–60 years, and >61 years. Maximum patients

belonged to 21–60 years of age (57.5%) and least patients belonged to <10 years of age (7.9%). Mean age of the patients was 36.69 ± 20.43 years while median age was 35 years. With respect to FAB type, i.e. M3 and non-M3, there was maximum number of patients from 21 to 60 years of age group in both types. Smoking history was present in 24.9% patients, but there was no history of pack-years smoked.

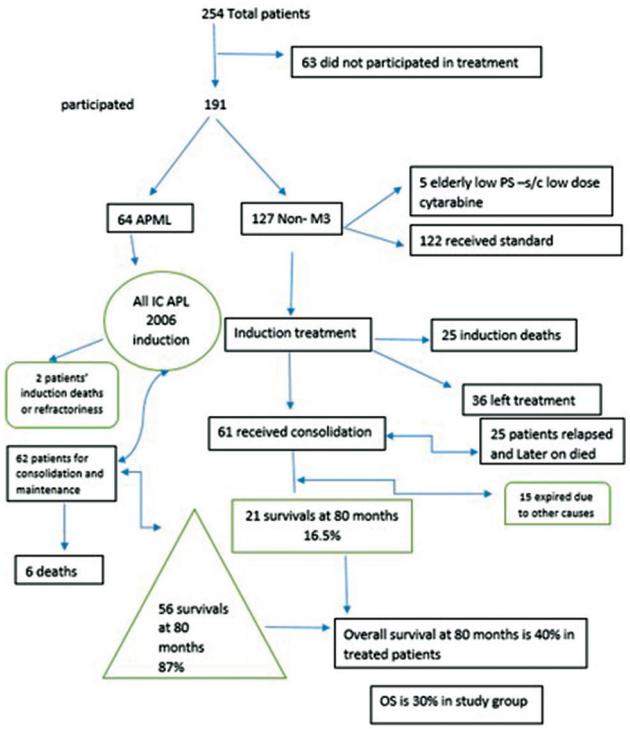

Figure 1: Study design

The most common presenting symptoms were related to features of symptomatic anemia which were seen in 44.9% of the patients followed by fever (37.8%), followed by bleeding. In M3, most common symptoms were related to bleeding, while in non-M3, most common symptoms were related to anemia. Rarer presentations included symptoms of chloromas which was seen in periorbital areas, parotid, and uterus. Other rarer presentations include symptoms of hearing loss and recurrent boils, and few were incidentally discovered when investigations were done for another reasons [Table 3]. The most common sign was pallor (57.4%), organomegaly (22.4%) followed by lymphadenopathy (16.1%). Chloromas were seen in only 1.2% of patients. Pallor continued to be the most common sign after dividing patients into M3 and non-M3, with organomegaly and chloromas more common in non-M3 [Table 4].

Among laboratory profile, mean hemoglobin was 5.84 ± 0.5 g/dl and median hemoglobin was 5 g/dl. In both M3 and non-M3, hemoglobin was in the range of 5–10 g/dl. Total leukocyte count (TLC) of more than 11 × 10^3/µl was seen in the majority of the patients. Mean TLC was 5.26 ± 1.17 × 10^3/µl and median was 2.7 × 10^3/µl. In M3, the most common presenting TLC was <4 × 10^3/µl, while in non-M3, the most common presenting TLC was >11 × 10^3/µl. Majority of the patients presented with thrombocytopenia with a platelet count of <50 × 10^3/µl. Mean platelet count was 31.96 ± 1.17 × 10^3/µl while median was 30 × 10^3/µl. The most common platelet count of <50 × 10^3/µl was seen in both M3 and non-M3. Peripheral blood film (PBF) in majority of the patients has blast percentage of more than

Table 3: Presenting symptoms

Symptoms	Number of- patients/ percentages	AML type		Total
		M3	Others	
Anemia	Count	6	28	34
	Percentage within AML type	10.3	24.1	19.5
Fever	Count	5	17	22
	Percentage within AML type	8.6	14.7	12.6
Bleeding	Count	16	2	18
	Percentage within AML type	27.6	1.7	10.3
Anemia+fever	Count	2	20	22
	Percentage within AML type	3.4	17.2	12.6
Anemia + bleeding	Count	11	12	23
	Percentage within AML type	19.0	10.3	13.2
Fever + bleeding	Count	8	10	18
	Percentage within AML type	13.8	8.6	10.3
Anemia + fever + bleeding	Count	7	14	21
	Percentage within AML type	12.1	12.1	12.1
Others	Count	3	13	16
	Percentage Within AML type	5.2	11.2	9.2
Total	Count	58	116	174
	Percentage within AML type	100.0	100.0	100.0

AML – Acute myeloid leukemia

Table 4: Presenting signs

Signs	Number of- patients/ percentages	AML type		Total
		M3	Others	
Pallor	Count	31	46	77
	Percentage within AML type	58.5	41.8	47.2
LAP	Count	0	2	2
	Percentage within AML type	0.0	1.8	1.2
Organomegaly	Count	2	2	4
	Percentage within AML type	3.8	1.8	2.5
Gingival hyperplasia	Count	1	0	1
	Percentage within AML type	1.9	0.0	0.6
Chloroma	Count	0	3	3
	Percentage within AML type	0.0	2.7	1.8
Pallor + LAP	Count	1	9	10
	Percentage within AML type	1.9	8.2	6.1
LAP + organomegaly	Count	0	2	2
	Percentage within AML type	0.0	1.8	1.2
Pallor + organomegaly	Count	7	18	25
	Percentage within AML type	13.2	16.4	15.3
Pallor + LAP + organomegaly	Count	1	12	13
	Percentage within AML type	1.9	10.9	8.0
Pallor + LAP + organomegaly + gingival hyperplasia	Count	3	6	9
	Percentage within AML type	5.7	5.5	5.5
Others	Count	2	3	5
	Percentage within AML type	3.8	2.7	3.1
Normal	Count	5	7	12
	Percentage within AML type	9.4	6.4	7.4
Total	Count	53	110	163
	Percentage within AML type	100.0	100.0	100.0

LAP – Leukocyte alkaline phosphatase; AML – Acute myeloid leukemia

50%. Mean of PBF blasts was 40.32% ± 0.8% and median was 39%.

AML was divided into different FAB types on the bone marrow morphology. Maximum cases (33%) had M3 type [Table 5 and Figure 2a, b]. Flow cytometry was available in 90 patients only and diagnostic yield was highest with CD13, CD33, CD117, and MPO. These markers were highly positive in approximately 90% of patients [Table 6]. Cytogenetic and molecular studies were available in 105 patients, and the most frequent cytogenetic abnormality found was of t(15; 17) [Table 7 and Figure 3]. Bone marrow blast percentage of more than 80% was seen in majority of patients; only one case of erythroleukemia was found. Mean bone marrow blast percentage was 73.3% ± 22.5% and median was 80.5%.

In our total of 254 patients, maximum patients were M3 phenotype, and around one-fourth of patients belonged to APML, proven by reverse transcription-polymerase chain reaction [Table 5]. To all patients of APML, the standard ICAPL-2006 protocol was used, with a dose of ATRA of 45 mg/m^2.Treatment was individualized on the basis of risk, low or high risk, depending upon TLC count of less or more than10,000/µl, respectively. Hence, of 254 patients, 25% (64) patients were APML and 190 (75%)

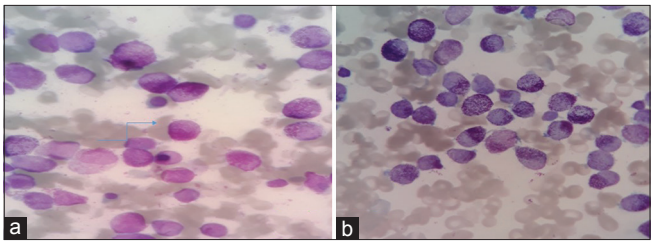

Figure 2: (a) Promyelocytes with granular cytoplasm. (b) With Auer rods

were non-M3, either phenotypically or non-t(15;17). Of 190 patients, only 127 (67%) patients received treatment. All non-M3 patients received standard treatment depending upon performance status and age. Only five patients received low-dose cytarabine, and rest 122 patients received standard 3:7 induction regimen. Cytarabine was given 200 mg/m^2 for 7 days with daunorubicin at a dose of 45 mg/m^2 or 60 mg/m^2. Remission was assessed on average of day 45 of APML protocol and day 28 of 3:7 protocol [Table 8]. There was no difference in outcome in different doses of daunorubicin. Around 2.2% of APML patients were refractory to initial treatment induction, 1.6% succumbed during induction, and 16.0% of non-M3 did not achieve CR with 20.8% induction deaths. Of 97 patients of non-M3 phenotype, only 61 patients were available

Table 5: Bone marrow morphology (FAB types)

FAB type	Frequency (%)
M0	8 (3.1)
M1	34 (13.4)
M2	58 (22.8)
M3	64 (25.2)
M4	8 (3.1)
M5	5 (2.0)
M6	8 (3.1)
M7	3 (1.2)
MDS progress to AML	6 (2.4)
Total	194 (76.4)
Unknown	60 (23.6)
Total	254 (100.0)

MDS – Myelodysplastic syndrome; AML – Acute myeloid leukemia

Table 6: Flow cytometry analysis of patients

Flow cytometry	Frequency (%)		Total
	Positive	Negative	
CD11b	24 (25.3)	71 (74.7)	95
CD11c	9 (9.7)	84 (90.3)	93
CD13	87 (87.9)	12 (12.1)	99
CD15	47 (50.0)	47 (50.0)	94
CD19	10 (12.8)	68 (87.2)	78
CD33	95 (95.0)	5 (5.0)	100
CD34	53 (54.6)	44 (45.4)	97
CD36a	16 (18.6)	70 (81.4)	86
CD41	3 (3.2)	91 (96.8)	94
CD45	88 (88.9)	11 (11.1)	99
CD56	10 (11.6)	76 (88.4)	86
CD61	2 (2.1)	92 (97.9)	94
CD64	42 (48.8)	44 (51.2)	86
CD113	4 (4.7)	81 (95.3)	85
CD117	83 (83.8)	16 (16.2)	99
CD123	40 (46.0)	47 (54.0)	87
MPO	97 (89.0)	12 (11.0)	109

MPO – Myeloperoxidase; CD – Cluster differentiation

to high-dose cytosine arabinoside consolidation of 3 g/m^2 [Table 9].

Effect of different variables on overall and event-free survival was studied. Sex had no significant impact, but age had significant impact on survival. There was a statistically significant relationship between age group and outcome ($P < 0.001$). Death was most common in the age group of >60 years (88.9%). The odds ratio of death was 8.0 (95% confidence interval [CI] 1.626, 39.354) times more in the age group of >60 years as compared to the age group of <10 years [Table 10]. Smoking of any kind also had significant impact on final outcome of patient [Table 11].

Among laboratory variables, hemoglobin level had no significant impact, but TLC and platelet count had statistically significant impact on survival of patient [Table 12] ($P < 0.05$). Any TLC higher than 11000/µl had bad outcome, especially in APML which is considered as high-risk APML, and platelet count <50,000/µl again had similar results. Peripheral blood blasts and overall survival had insignificant relation, but bone marrow blast percentage had significant relation with outcome of patients ($P < 0.001$).

Figure 3: Conventional karyotype revealing t(8;21)

Bone marrow was evaluated for morphology and different FAB types were assigned. A total of 64 M3 and 127 non-M3 phenotypes were seen. The outcome in M3 was far better than non-M3 subtypes. Overall survival was 87% in M3 versus 16.5% in non-M3, which is statistically significant [Table 13] ($P < 0.001$). Again, it was proved that t (15;17) in cytogenetics had superior outcome which is statistically significant ($P < 0.001$).

CR rate in non-M3 AML was 63.2% while in M3 was 96.2%. Relapse rate was 12.8% in M3 and 26.2% in non-M3. Overall, there were around 40% of patients alive at the time of analysis of data, i.e. December 30, 2016. After analyzing any event, i.e. death or relapse with respect to months passed since diagnosis up to the last follow-up, i.e. December 30, 2016, it was found that 75% of patients in M3 group had no event at 80 months, while in non-M3, the same was 16%. Overall survival was almost similar to event-free survival because no patient underwent allogenic stem cell transplant due to the lack of facility, and moreover, majority of patients did not opt for the second-line treatment in view of grim prognosis. Cox regression analysis was done with "time to event (in months)" as the time variable and cytogenetics, age, TLC, platelet count, and M-type as categorical covariates. There was a statistically significant relationship of cytogenetics with time to event. The hazard ratio (HR) was significantly lower for the "good + t(15;17)" category as compared to "normal" category (HR = 0.020, 95% CI 0.002–0.179) [Figure 4]. Age, TLC, platelet count, and M-type were not

significantly related to time to event in the multivariate analysis. Overall survival for intended to treat patients was 40% and for study group was 30%; overall survival for M3 group was 87% and for non-M3 group was 16.5% [Figure 5a and b].

Discussion

AML is a heterogeneous hematologic malignancy, characterized by clonal expansion of myeloid blasts in the bone marrow, peripheral blood, and other tissues. It is the most common form of acute leukemia among adults and accounts for the largest number of deaths from leukemia in the United States. Our study is based on the hospital cancer registry, irrespective of the age of the patient. Most of the studies in patients with AML have included all of the FAB AML types, except for APML, while our study included all the AML, irrespective of FAB type. Mean age of our study patients is 36.69 ± 20.43 years and median age of 35 years, which is contrary to the median age mentioned in the current NCCN 2016 guidelines, i.e. 67 years. On further analysis, we found worst outcome in the age group of >60 years which is very well-accepted fact across all the studies. Only 13.4% of the patients in our study had age >60 years and they had worst outcome with survival rate of 11.1%. As reported by Appelbaum *et al*., percentage of patients with favorable cytogenetics dropped from 17% in patients younger than 56 years to only 4% in patients older than 75 years.[13] Pouls *et al*. published the data of 94 AML cases in which the major shortcoming was of no flow cytometry used in the diagnosis. Mean age of the studied subjects was 33.8 years with the most common presenting feature being pallor followed by bleeding and fever.[14]

As far as sex distribution of our patients is concerned, it was consistent with other studies as there was nonsignificant difference in the numbers of males and females. Males comprised 55.9% with a male-to-female ratio of 1.2. In CALGB, 8461 (47%) of patients were females. Ghosh *et al*. reported male preponderance, with a male-to-female ratio of 2.5:1.[4]

Smokers constituted 26.4% of studied patients and the outcome was significantly associated with smoking (P = 0.047) although there are no data regarding pack-years. Varadarajan *et al*. reported the similar difference in outcome with respect to overall survival.[15]

Clinical features of our patients were similar to other studies such as presenting symptom being the most commonly symptomatic anemia, followed by fever and bleeding. Lymphadenopathy was seen in 16.1% and organomegaly in 22.4%. Ghosh *et al*. reported lymphadenopathy in 36% of cases and organomegaly in 26% of cases. Hoffman has reported the presence of splenomegaly in 50% of his cases. Chloromas were seen in 1.2% of patients and the sites included periorbital tissues, uterus, and parotid. Geographic variations have been reported in the distribution of extramedullary leukemia and are more frequently reported from the African countries such as Uganda, Egypt, and

Table 7: Cytogenetics and molecular profile

	Frequency (%)
t(15;17)	57 (22.4)
t(8;21)	3 (1.2)
Complex	5 (2.0)
Del 5	1 (0.4)
Del 7	2 (0.8)
AMPL variant	4 (1.6)
Normal	24 (9.4)
Trisomy 21	1 (0.4)
Del 11	1 (0.4)
t(11;12)	1 (0.4)
t(9;11)	1 (0.4)
Del 12	1 (0.4)
Inv(16)	3 (1.2)
t(1;7)	1 (0.4)
Total	105 (41.3)
Unknown	149 (58.7)
Total	254 (100.0)

AMPL – Acute promyelocytic leukemia

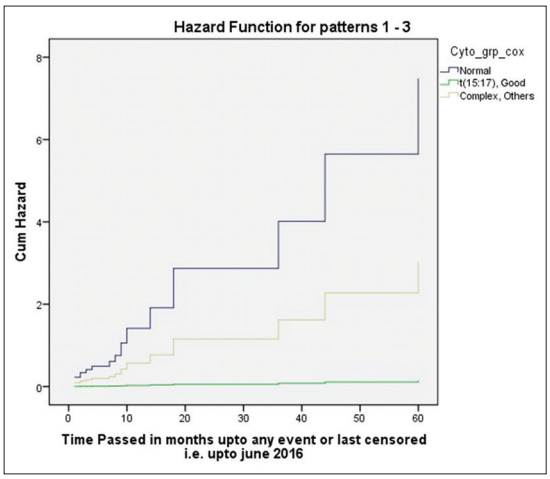

Figure 4: Multivariate analysis revealing lowest hazard for acute promyelocytic leukemia (M3)

Table 8: Phenotype and remission status

Phenotype	Number of patients	Treatment received	No treatment received	Percentage of patients	Valid percentage	CR status (%)
M3 or APML	64	64	0	25	100	96.2
Non-M3	190	95	95	75	50	63.2

CR – Complete remission; APML – Acute promyelocytic leukemia

Turkey. Shome *et al*. have reported an incidence of 17.9% for orbital granulosarcoma occurring in patients with acute nonlymphocytic leukemia. It is commonly associated with the AML-M4 subtype. Granulocytic sarcomas have also been observed in the AML-M2 subtype with t(8; 21) and leukocytosis. However, extramedullary leukemia is reported to adversely affect the hematologic remission rate and overall survival in patients with t(8; 21).[4]

The most common FAB type in our study population was found to be M3 type which is contrary to other studies. M3 constituted 25.2% of cases and M2 constituted 22.8%. Most of the studies have reported M2 as the most common subtype and few as M1 or M4.

Immunophenotyping has become an important diagnostic tool in establishing the diagnosis and classification of acute leukemia. The leukemic cells in all cases of M0 through M5 commonly express various combinations of CD13, CD33, CD65, CD117, and MPO. However, except for the monocytic markers and megakaryocyte-associated markers, CD41a, CD61, and CD42b antigens, other myeloid-associated markers (CD11b, CD11c, CD13, CD33, CD15, CD65, CD66, and CD117) are not useful in distinguishing the different subtypes of AML. Early myeloblasts express CD34 and human leukocyte antigen-D related (HLA-DR), but these are lost by the promyelocyte stage. Borowitz *et al*. have reported a higher positivity of CD34 (45%) in the more immature leukemias and a strong association with loss or partial deletion of chromosome 7 and 5. Callea *et al*. have found a strong correlation between HLA-DR positivity and AML-M4 and M5 subtypes. The author also reports a higher percentage of CRs in HLA-DR-negative cases as compared to the HLA-DR-positive ones. The AML-M1 subtype is usually associated with expression of CD13, CD33, CD34, CD65, CD117, and HLA-DR in variable combinations. The leukemic blasts in cases of t(8;21)(q22:q22)-associated AML-M2 have a distinct immunophenotype. They exhibit CD34, CD65, and HLA-DR, but CD33 and CD13 expression is very weak or sometimes may be absent. Many of them weakly express CD19 and less commonly CD56. Incidence of positivity for the stem cell-associated antigen, CD34 and HLA-DR, in t(8;21) AML cells was significantly higher than those in other AML with granulocytic maturation such as AML-M2 without t(8;21)

Table 9: Consolidation status of non-M3 patients

Number of Ara-C cycles in consolidation	Number of patients available (%)	Valid percentage
3 cycles	35 (13.8)	50.0
4 cycles	18 (7.1)	33.1
<3 cycles	1 (0.4)	1.4

Table 10: Impact of age on final outcome

		Number of patients/ percentages	Outcome		Total	*P* Value (Chi-square test)	Odds Ratio (95% CI)
			Alive	Dead			
Age	<10 years	Count	7	7	14	<0.001	
		% within Age	50.0%	50.0%	100.0%		
	11-20 years	Count	28	14	42		0.500 (0.146, 1.708)
		% within Age	66.7%	33.3%	100.0%		
	21-60 years	Count	58	50	108		0.862 (0.283, 2.626)
		% within Age	53.7%	46.3%	100.0%		
	>60 years	Count	3	24	27		8.000 (1.626, 39.354)
		% within Age	11.1%	88.9%	100.0%		
Total		Count	96	95	191		
		% within Age	50.3%	49.7%	100.0%		

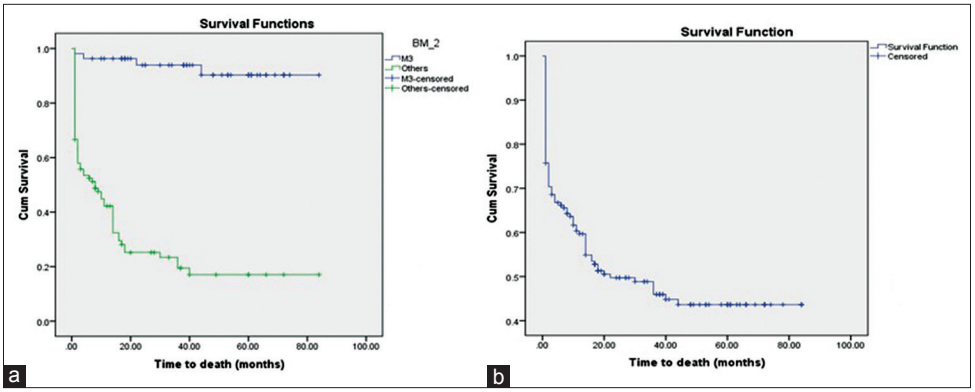

Figure 5: (a) Overall survival curve with 87% and 16% survival at 80 months for M3 and non-M3 types, respectively. (b) Overall survival was 40% at 80 months

Table 11: Impact of smoking on final outcome

Smoking	Number of- patients/ percentages	Outcome		Total	*P*
		Alive	Dead		
Yes	Count	17	29	46	0.043
	Percentage within smoking	37.0	63.0	100.0	
No	Count	79	66	145	
	Percentage within smoking	54.5	45.5	100.0	
Total	Count	96	95	191	
	Percentage within smoking	50.3	49.7	100.0	

Table 12: Impact of total leukocyte count on outcome

TLC	Number of- patients/ percentages	Outcome		Total	*P*
		Alive	Dead		
<4 ($\times10^3$/µl)	Count	44	19	63	0.008
	Percentage within TLC	70.2	29.8	100.0	
4-11 ($\times10^3$/µl)	Count	23	21	44	
	Percentage within TLC	51.5	48.5	100.0	
>11 ($\times10^3$/µl)	Count	34	50	84	
	Percentage within TLC	40.3	59.7	100.0	
Total	Count	101	90	191	
	Percentage within TLC	52.8	47.2	100.0	

TLC – Total leukocyte count

Table 13: FAB type and outcome of patient

FAB type	Number of- patients/ percentages	Outcome		Total	*P*
		Alive	Dead		
M0	Count	1	4	5	<0.001
	Percentage within BM	20.0	80.0	100.0	
M1	Count	6	23	29	
	Percentage within BM	20.7	79.3	100.0	
M2	Count	18	27	45	
	Percentage within BM	40.0	60.0	100.0	
M3	Count	56	8	64	
	Percentage within BM	93.4	6.6	100.0	
M4	Count	3	3	6	
	Percentage within BM	50.0	50.0	100.0	
M5	Count	1	2	3	
	Percentage within BM	33.3	66.7	100.0	
M6	Count	1	6	7	
	Percentage within BM	14.3	85.7	100.0	
M7	Count	0	3	3	
	Percentage within BM	0.0	100.0	100.0	
MDS progress to AML	Count	0	5	5	
	Percentage within BM	0.0	100.0	100.0	
Total	Count	86	81	167	
	Percentage within BM	53.0	47.0	100.0	

BM – Bone marrow; MDS – Myelodysplastic syndrome; AML – Acute myeloid leukemia

and AML-M3. The combination of CD markers which was present across all subtypes of AML in more than 50% of patients included CD13, CD33, CD45, CD117, and MPO.

Cytogenetic analysis was available in 105 patients. The most common cytogenetic abnormality found was t(15;17) seen in 54.3% followed by normal cytogenetics seen in 22.9%, while other good-risk cytogenetics such as t(8;21) and inv (16) was seen in 5.8% of patients. Our findings were consistent with the findings of Cheng *et al*. and Ayesh *et al*., who also had the most common cytogenetic abnormality in the form of t(15:17).[16,17]

Considering present treatment options of newly diagnosed AML except APML, induction consists of daunorubicin, idarubicin, and cytarabine based. Induction consists of daunorubicin with a dose range of 45–90 mg/m^2. Recent studies have clearly shown dose of 60 mg/m^2 better than 45 mg/m^2, and then further, Burnett *et al*. have clearly shown 60 mg/m^2 better than 90 mg/m^2 in terms of same CR and lower 60-day mortality. Postinduction treatment is decided on the basis of risk stratification by cytogenetic and molecular markers. Those who require chemotherapy as consolidation generally are given 3–4 cycles of HiDAC. In our study, no significant difference in outcome with different doses of daunorubicin and cytarabine with outcome in induction was found. Further, in consolidation, no significant relationship with different number of cycles of cytarabine in consolidation was found.

Analysis of survival with respect to laboratory parameters including white blood cells, platelets, and FAB type is found to be statistically significant. Best survival was found in M3 type as expected.

After analyzing survival on Kaplan–Meir curve, around 40% of patients had no event at 4 years. After analyzing event with respect to AML M3 and non-M3 separately at 5 years, 75% of patients in M3 and 16% in non-M3 had no event. After looking at the curve carefully, majority of the patients in non-M3 had an event by around 20 months since diagnosis. Hence, practically, it means that a non-M3 patient surviving beyond 2 years can be declared as cured. Overall survival was almost similar to event-free survival because there was no bone marrow transplant available at the time of relapse. Only AML M3 patients went for the second line of chemo and rest did not opt for the treatment considering grim prognosis. Schlenk *et al*. reported the 4-year survival rate in normal cytogenetic population of around 43%.[18]

Conclusion

In our study, males outnumbered females and most of our patients were in 20–60 years of age group. The better prognosis was in patients who were in the second decade of life. TLC and platelet count had significant impact on survival of patient. Bone marrow morphology of M3 type has extremely good prognosis and was most common FAB type seen in our study. Flow cytometric markers such as CD15, CD33, CD117, and MPO had positivity among 90% of patients. Overall survival is around 40% in whole-study group, 87% in APML group, and 16.5% in non-M3 group. There are still unmet needs in managing

the non-M3 patients in resource-constraint countries where allogenic transplant and newer drugs have the least access. Further, there is long way to go in the future to improve supportive care treatment in APML and pushing newer cheap molecules in treatment paradigm for non-M3 patients.

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Conflicts of interest

There are no conflicts of interest.

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