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Cytogenetics' impact on the prognosis of acute myeloid leukemia

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Abstract:

INTRODUCTION: Acute myeloid leukemia (AML) is a group of disorders characterized by a spectrum of clinical, morphological, immunophenotypic, and associated chromosomal abnormalities. The identification of cytogenetic abnormalities at diagnosis is important for the evaluation of the response to therapy and the identification of an early reemergence of disease.

MATERIALS AND METHODS: Newly diagnosed cases of AML were included in the study. Diagnosis of AML was based on morphology on bone marrow (BM) aspirates, cytochemistry, and flow cytometric immunophenotyping. Chromosomal analysis was performed on BM by short-term unstimulated cultures using standard cytogenetic technique.

RESULTS: There were 25 males and 13 females with age group between 15 and 64 years. Cytogenetic analysis of these cases showed normal karyotype in 10 (26.3%) cases and abnormal karyotype in 28 (73.6%) cases. Cytogenetic finding in AML was divided into three groups: favorable risk, intermediate risk, and unfavorable risk. Patients in the standard risk group responded well to the chemotherapy while patients with intermediate and unfavorable karyotype had relapsed.

CONCLUSION: We recommend that cytogenetics should be performed routinely in all cases of AML. A correlation must be done with various biochemical and hematological parameters, immunophenotyping, and BM morphology. Molecular studies must be integrated with cytogenetic studies for risk stratification at diagnosis to improve therapeutic strategies.

Key words:

Acute myeloid leukemia, cytogenetics, immunophenotyping

Introduction

Acute myeloid leukemia (AML) is the most common acute leukemia in adults, resulting from the clonal expansion of myeloid blasts in the peripheral blood (PB), bone marrow (BM), or other tissue.^[1]

Cytogenetically, AML is a very heterogeneous disease, with more than 160 recurrent structural chromosomal abnormalities. Cytogenetic evaluation of myeloid disorders is useful for diagnosis, to identify a proliferation as clonal or not, especially when there is diagnostic dilemma between a neoplastic or a reactive process to

decide the therapy. This includes the choice of a specific treatment protocol as well as the decision and the timing of hematopoietic stem cell transplant. The identification of cytogenetic abnormalities at diagnosis is also important for the evaluation of the response to therapy and the identification of an early reemergence of disease.^[2]

However, several pretreatment factors such as age, performance status, leukocytes count, and karyotype predict the outcome, but karyotype certainly is the most important prognostic factor for the rate of complete remission (CR), overall survival (OS), and disease-free survival.^[3]

Cytogenetic test may help predict how cancer will respond to treatment and allow

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physician to plan more effective therapy. This study was performed to determine the incidence of chromosomal abnormalities in patients with AML and to correlate specific chromosomal abnormalities with clinical and laboratory parameters in these patients.

Materials and Methods

All 38 newly diagnosed cases of AML seen at the Department of Haematology, All India Institute of Medical Sciences, between August 2010 and July 2012 were included in the study. Diagnosis of AML was based on morphology on BM aspirates (as defined by the FAB classification), cytochemistry, and flow cytometric immunophenotyping in all patients with age ≥ 15 years.

Immunophenotyping was performed on flow cytometer BD FACS Canto II (Becton Dickinson, San Jose, CA, USA). Specimens used were 2 ml EDTA PB and/or BM aspirate. The analysis was performed on FACS Diva software using 6-color protocol.

Chromosomal analysis was performed on pretreatment BM, or rarely PB (blast count $\geq 20\%$) (when marrow is not unavailable) on short-term unstimulated cultures (with or without colcemid) using standard cytogenetic technique.^[4] G-banded chromosomes were classified according to the International System of Human Cytogenetic Nomenclature.^[5] At least 20 metaphases were analyzed in each patient.

Results

During this period, 38 newly diagnosed cases of AML were analyzed. There were 25 males and 13 females (ratio 1.9:1) with age group between 15 and 64 years (mean 30.6 years). All patients had pallor, while 26 (68.4%) patients had fever and 11 (28.9%) patients had bleeding manifestation in the form of petechiae, except 1 (2.6%) patient who had hematuria. On examination, bony tenderness was present in 8 (21.0%) patients and 2 (5.2%) patients had gum hypertrophy. Hepatomegaly was present in 19 (50.0%) patients, splenomegaly in 11 (28.9%) patients, and 4 (10.5%) patients had lymphadenopathy [Table 1].

On investigations, hemoglobin ranges from 2.3 to 10.6 gm% (mean 6.5 gm%), total leukocyte count $1.5\text{--}179 \times 10^6/\mu\text{l}$ (mean $30.4 \times 10^6/\mu\text{l}$), and platelet count $5.0\text{--}150 \times 10^6/\mu\text{l}$ (mean $57.3 \times 10^6/\mu\text{l}$). In BM blasts, count varies from 20% to 92% (mean 61%). On cytochemistry, all blasts were positive for myeloperoxidase (MPO) and Sudan Black B. In AML, M4 and M5 blasts were positive for nonspecific esterase.

On flow cytometry, the blasts were positive in all cases for CD13, CD33, aMPO, CD34, and HLA-DR, except in

Table 1: Clinical profiles of acute myeloid leukemia patients

Age/sex	Fever	Pallor	Bleeding manifestation	Liver	Spleen	LN
50/male	P	P	A	NP	1 cm	NP
25/female	P	P	P	4 cm	2 cm	NP
16/female	P	P	A	NP	NP	NP
15/male	A	P	A	2 cm	8 cm	P
21/female	P	P	A	6 cm	NP	NP
22/male	P	P	P	1 cm	NP	NP
32/male	P	P	A	NP	NP	P
61/male	A	P	P	NP	NP	NP
30/male	A	P	P	2 cm	NP	NP
15/male	P	P	P	1 cm	NP	NP
34/male	P	P	P	NP	NP	NP
41/male	A	P	A	NP	NP	NP
32/female	P	P	P	1 cm	NP	NP
64/male	P	P	A	1 cm	NP	NP
10/female	P	P	A	NP	NP	NP
50/female	P	P	A	NP	NP	NP
24/male	A	P	A	NP	2 cm	NP
28/male	A	P	A	NP	NP	NP
16/male	P	P	P	NP	NP	NP
37/female	P	P	A	3 cm	NP	NP
25/male	P	P	A	4 cm	NP	P
36/male	A	P	A	1 cm	NP	NP
18/male	P	P	A	NP	NP	NP
27/male	P	P	A	NP	NP	NP
43/male	P	P	A	2 cm	3 cm	NP
23/female	A	P	A	NP	2 cm	NP
15/male	P	P	A	2 cm	1 cm	NP
27/male	P	P	A	NP	NP	NP
32/female	A	P	A	2 cm	5 cm	NP
24/male	P	P	A	NP	NP	NP
23/female	P	P	A	3 cm	2 cm	P
18/male	A	P	P	1 cm	NP	NP
44/male	P	P	A	NP	NP	NP
40/female	P	P	P	2 cm	1 cm	NP
45/female	A	P	A	NP	NP	NP
35/female	P	P	A	NP	NP	NP
19/male	A	P	A	3 cm	2 cm	NP
42/male	P	P	P	1 cm	NP	NP

P = Present, A = Absent, P = Palpable, NP = Not palpable, LN = Lymph node

2 (5.2%) cases of acute promyelocytic leukemia in which blasts and abnormal promyelocytes were negative for HLA-DR and CD34. The blasts were positive for CD64 and CD117 in 7 (18.4%) and 8 (21.0%) cases, respectively. There was aberrant expression of CD10, CD19, and CD79a in 5 (13.1%) cases, 1 (2.6%) case, and 2 (5.2%) cases, respectively.

Cytogenetic analysis of these cases showed normal karyotype in 10 (26.3%) cases and abnormal karyotype in 28 (73.6%) cases. Cytogenetic finding in AML was divided into three groups: favorable risk, intermediate risk, and unfavorable risk. There were 12 (31.5%) cases in the favorable risk group and cytogenetic abnormality includes cases with the t(8;21), inv(16), and the t(15;17).

There were 9 (23.6%) cases in the standard risk group which includes cases with trisomy 8, trisomy 6,-Y, del(12p), and normal karyotype. The unfavorable group includes 7 (18.4%) cases with del(7q), t(3;5), t(6;9) and complex karyotypes [Table 2].

Patients in the standard risk group responded well to the chemotherapy while patients with intermediate and unfavorable karyotype had relapsed and most of the patients are lost to follow-up while two patients are on palliative therapy.

Discussion

AML is a group of disorders characterized by a spectrum of clinical, morphological, immunophenotypic, and associated chromosomal abnormalities.^[6] The classification of AML has evolved from the primarily morphologic and cytochemical system of the early French-American-British (FAB) cooperative group proposal to the systems that consider the results of cytogenetic studies.^[7,8]

Although morphological evaluation of BM aspiration and biopsy remains important for the diagnosis of AML, it is clear that the presence or absence of specific cytogenetic abnormalities and acquired genetic mutations remain as a cornerstone in predicting prognosis (favorable, intermediate, and unfavorable risk groups) as well as guiding the treatment.^[9,10]

The advantage of cytogenetic analysis is that it has the intrinsic ability to detect any structural or numerical aberration, novel, and uncharacterized abnormalities. Chromosomal aberrations are seen in 90% of AML patients. The recent WHO classification has also stressed on the importance of cytogenetic abnormalities and multilineage dysplasia in the subtyping of leukemias.^[1,11,12]

Consistent with the findings of other international reports, in our study, 73% of the patients showed karyotypic abnormalities. Clonal chromosomal aberrations are not detected in 26.3% of AML patients.^[10]

In our study, younger patients more frequently had balanced translocations such as t(8;21) while complex karyotype was found in elderly patients similar to the literature. Probably different genetic mechanisms are involved in the pathogenesis of AML, and these mechanisms might occur at different frequencies as age increases.^[9]

About 26.3% of AML patients in our study had a normal karyotype by cytogenetic analysis. Studies from other countries have reported a normal karyotype in AML

with a frequency of about 35%–45%. The cytogenetically normal karyotype in AML is considered an intermediate cytogenetic risk group because of varying response to treatment, achievement of CR, and relapse rate. These patients should be investigated for molecular genetics alterations.^[9,10,13]

Missed chromosome aberrations in AML with a normal karyotype could be due to the inability of the abnormal clone with aneuploidy to proliferate *in vitro*, poor quality of the chromosome morphology, and the G-banding resolution to detect aberration or due to cryptic rearrangements. In one case of inv16, we had a doubt because of poor morphology which later confirmed by fluorescence *in situ* hybridization.

We also assessed the role of immunophenotyping and cytogenetics and their clinicopathological correlation with various hematological parameters and found a statistically significant correlation with various parameters and supported that expression of certain antigens and abnormal karyotypes correlate with a poor prognosis in AML. Two cases with aberrant expression of CD79a were in unfavorable group while CD19 was expressed in favorable risk group. Expression of CD10 was present in all the risk groups in our study.

Limitation of our study was the numbers of cases with cytogenetic analysis were few to arrive at any meaningful conclusion and survival analysis of these patients could not be obtained as many were lost to follow-up during the study.

Conclusion

We recommend that cytogenetics should be performed routinely in all cases of AML. A correlation must be done with various biochemical and hematological parameters, immunophenotyping, and BM morphology. Molecular studies must be integrated with cytogenetic studies for risk stratification at diagnosis to improve therapeutic strategies.

Cytogenetic findings should be integrated into a prognostic index applicable in risk-directed therapy decision-making for younger patients with AML. The patients with poor cytogenetics the chance of cure is very low even with allogenic stem cell transplant. Hence, a clear need exists for a large prospective studies evaluating association between karyotype and clinical outcome.

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Table 2: Hematological, immunophenotypic, and cytogenetics profile of acute myeloid leukemia patients

Hb g%	TLC ×10 ⁶ /μl	Platelets ×10 ⁶ /μl	Blasts (%)	Diagnosis	CG	Cytochemistry	Flow cytometry
8.3	3.3	100.0	20%	AML	46, XY[20]	SBB, MPO+	CD33, CD13, aMPO, HLA-DR, CD34
2.3	2.0	75.0	50	APML	46, XX, t (15;17)(q22;q21)[20]	SBB, MPO+	CD33, CD13, aMPO, CD10, CD19, CD34, CD64, CD117
2.7	28.4	10.0	60	AML	46, XX, t (8;21)(q22;q22)[20]	SBB, MPO+	CD33, CD13, aMPO, HLA-DR, CD34, CD117
7	160.0	300.0	65	AML-M5	47, XY,+8, del (11)(q23)[20]	SBB, MPO+, NSE+	CD33, CD13, aMPO, HLA-DR, CD34, CD117, CD10, CD79a
5.4	56.3	85.0	88	AML M5	47, XX,+6[12]/46, XX[8]	MPO, SBB, NSE+	CD33, CD13, aMPO, HLA-DR, CD34, CD64, CD10
3.4	24.5	49.0	33	AML M4	46, XY, inv (16)(p13q22)[20]	MPO, SBB+, NSE+	CD33, CD13, aMPO, HLA-DR, CD34, CD117
10.6	88.0	100.0	90	AML	45, XY, inv (3)(q21q26)[19]	MPO, SBB+	CD33, CD13, aMPO, HLA-DR, CD34
6.1	14.5	15.0	30	AML	45, XY, t (8;21)(q22;q22),-Y[20]	MPO, SBB+	CD33, CD13, aMPO, HLA-DR, CD34
9	50.0	78.0	45	AML	46, XY, del (3)(q26)[10]/46, XY[10]	MPO, SBB+	CD33, CD13, aMPO, HLA-DR, CD34
7.8	23.0	12.0	80	AML	46, XY[20]	MPO, SBB+	CD33, CD13, aMPO, HLA-DR, CD34
8.1	65.4	42.0	90	AML	46, XY, t (3;5)(q25;q34)[20]	MPO, SBB+	CD33, CD13, aMPO, HLA-DR, CD34
8.2	1.5	150.0	70	AML	46, XY[20]	MPO, SBB+	CD33, CD13, aMPO, HLA-DR, CD34
6	5.8	10.0	92	AML	46, XX, t (9;11)(p21;q23)[20]	MPO, SBB+	CD33, CD13, aMPO, HLA-DR, CD34
10.1	179.0	47.0	50	AML	46, XY[20]	MPO, SBB+	CD33, CD13, aMPO, HLA-DR, CD34
3.5	5.5	10.0	54	AML	46, XX[20]	MPO, SBB+	CD34, HLA-DR, CD33, CD13, aMPO
6.4	5.2	150.0	72	AML-M1	46, XX[20]	MPO, SBB+	CD33, CD13, aMPO, HLA-DR, CD34
4.2	6.4	17.0	53	AML	46, XY, del (12)(p12)[20]	MPO, SBB+	CD33, CD13, aMPO, HLA-DR, CD34
4.6	3.9	20.0	20	AML-M6	46, XY[20]	MPO, SBB+	CD33, CD13, aMPO, HLA-DR, CD34
9	1.9	5.0	80	APML	46, XY, t (15;17)(q22;q21)[20]	MPO, SBB+	CD33, CD13, aMPO, HLA-DR, CD34
4	5.6	60.0	33	AML	46, XY[20]	MPO, SBB+	CD33, CD13, aMPO, HLA-DR, CD34
5	4.8	20.0	40	AML M4	46, XY, inv (16)(p13q22)[20]	MPO, SBB+, NSE+	CD33, CD13, aMPO, HLA-DR, CD34, CD64
8.4	8.6	120.0	20	AML	46, XY[20]	MPO, SBB+	CD33, CD13, aMPO, HLA-DR, CD34
6.5	15.5	53.0	80	APML	46, XY, t (15;17)(q22;q21)[20]	MPO, SBB+	CD33, CD13, aMPO, HLA-DR, CD34, CD13, CD33
7.8	8.7	25.0	43	AML M4	46, XY, inv (16)(p13q22)[20]	MPO, SBB+, NSE+	CD33, CD13, aMPO, HLA-DR, CD34, CD64
5.2	14.0	52.0	80	AML-M5	46, XY[20]	SBB, MPO+, NSE+	CD33, CD13, aMPO, HLA-DR, CD34, CD64, CD117
4.8	8.8	100.0	70	AML	46, XX, t (8;21)(q22;q22)[20]	SBB, MPO+	CD33, CD13, aMPO, HLA-DR, CD34
6.0	9.2	78.0	54	AML-M4	46, XY, ?inv (16)(p13;q22)[20]	SBB, MPO+, NSE+	CD33, CD13, aMPO, HLA-DR, CD34, CD64, CD117

Contd...

Table 2: Contd...

Hb g%	TLC ×10 ⁶ /μl	Platelets ×106/μl	Blasts (%)	Diagnosis	CG	Cytochemistry	Flow cytochemistry
3.8	4.5	35.0	68	AML	46, XY, del (12)(p12)[20]	SBB, MPO+	CD33, CD13, aMPO, HLA-DR, CD34, CD10
7.1	3.9	82.0	80	AML	45, XX, dic (9;12)(q10;q10)[20]	SBB, MPO+	CD33, CD13, aMPO, HLA-DR, CD34
8.2	6.4	90.0	35	AML	47, XY,+8[12]/46, XY[8]	SBB, MPO+	CD33, CD13, aMPO, HLA-DR, CD34
4.5	5.4	76.0	49	AML-M5	46, XY, del (11)(q23)[20]	SBB, MPO+, NSE+	CD33, CD13, aMPO, HLA-DR, CD34, CD64, CD117
5.6	142.0	20.0	90	APML	46, XY, t (15;17)(q22;q21)[20]	SBB, MPO+	CD33, CD13, aMPO, HLA-DR, CD34
8.8	18.2	35.0	72	AML	46, XY, del (3)(q21q26)[20]	SBB, MPO+	CD33, CD13, aMPO, HLA-DR, CD34, CD79a
7.0	5.8	18.0	88	APML	46, XY, t (15;17)(q22;q21)[20]	SBB, MPO+	CD33, CD13, aMPO, CD34
8.4	65.0	40.0	57	AML	45, XX,-7[8]/46, XX[12]	SBB, MPO+	CD33, CD13, aMPO, HLA-DR, CD34
9.2	28.3	68.0	65	AML	46, XX, t (8;21)(q22;q22)[20]	SBB, MPO+	CD33, CD13, aMPO, HLA-DR, CD34
7.9	50.4	60.0	82	AML	45, XY,-7, del (12)(p12)[20]	SBB, MPO+	CD33, CD13, aMPO, HLA-DR, CD34, CD117
6.5	24.1	20.0	80	AML	46, XY, t (6;9)(p12;q34)[20]	SBB, MPO+	CD33, CD13, aMPO, HLA-DR, CD34, CD10

AML = Acute myeloid leukemia, APML = Acute promyelocytic leukemia, SBB = Sudan Black B, MPO = Myeloperoxidase, NSE = Nonspecific Esterase, Hb = Hemoglobin, TLC = Total leukocyte count, CG = Cytogenetics

Conflicts of interest

There are no conflicts of interest.

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