

Significance of Cytogenetic Abnormalities in Acute Myeloid Leukaemia

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Abstract

Objective: To evaluate the role of karyotype in acute myeloid leukaemia (AML) as a predictor of response to induction chemotherapy.

Methods: A cross-sectional study was carried out at the department of Pathology and Oncology, Aga Khan University Karachi from January 2003 to January 2005. Newly diagnosed patients with denovo AML admitted to the hospital were included in the study. Diagnosis of AML was based on FAB criteria, immunophenotyping and cytogenetic studies. They were treated according to standard protocols (combination of anthracycline and cytarabine -3+7) and those who had acute promyelocytic leukaemia additionally received all- trans retinoic acid (ATRA).

Results: A total of 56 patients were enrolled, 4 were excluded due to inadequate cytogenetic analysis and the remaining patients entered the study protocol. There were 32 males and 20 females with mean age of 31.3 years (range 9 months to 73 years). Thirty-five (67.3%) patients had normal karyotype while 17 (32.7%) were found to have cytogenetic abnormalities. Eleven patients did not receive treatment at our hospital. Half of the (51.2%) patients out of remaining 41 achieved complete remission on bone marrow examination after receiving induction chemotherapy. In favourable risk group 3/3 (100%) achieved complete remission (CR) while 15/32 (46.9%) in intermediate risk group and 3/6 (50%) in unfavourable risk group. There was low CR rate in patients with high white cell counts.

Conclusion: The frequency of cytogenetic abnormalities in AML and response to induction chemotherapy was low when compared with international data possibly due to the small sample size. However, there was a clear difference in CR rates between favourable and unfavourable risk groups (JPMA 56:9;2006).

Introduction

Chromosomal abnormalities are valuable in the diagnosis and prognosis of different types of leukaemia and lymphoma. Many of these abnormalities are uniquely associated with specific histologic or immunologic subtypes of malignant haematological disorders.¹ However, presentation cytogenetics is widely recognized as one of the most important prognostic determinants in acute myeloid leukaemia (AML).² Substantial heterogeneity exists among patients with acute myeloid leukaemia which can be detected morphologically³ and with improved cytogenetic techniques.⁴ During the last two decades the clinical importance of both cytogenetic and molecular genetic analysis has become increasingly important in determining prognosis in AML. The identification of specific chromosomal abnormalities and their correlation with cytomorphologic features, immunophenotype and clinical outcome have led to new understanding of AML as a heterogeneous disease.⁵ Recently in the new classification of haematological malignancies by World Health Organization (WHO), specific cytogenetic abnormalities have been used to help define distinct disease entities among myeloid disorders.⁶

Several factors have been described to have prognostic significance in AML like patient characteristics such as age, performance status, adequacy of organ function and disease characteristics and at that same time, the clinical significance of cytogenetic aberration has become increasingly appreciated.⁷ Certain changes are highly correlated with particular FAB subgroups of AML, such as t(8;21) and M2, t(15;17) and M3, inv16 and M4 Eo, t(9; 11) and M5a, and t(1; 22) and M7.⁸ Recent reports suggest that the use of high resolution methods to study the banding pattern in extended chromosomes discloses that 90% of patients have an abnormal karyotype.⁴ To date, over 160 structural chromosome abnormalities have been reported in hematological malignancies.⁹

These recurring abnormalities have an important and independent impact on the prognosis, and they may influence the management of disease. New techniques such as fluorescence in situ hybridization (FISH), Southern blot, polymerase chain reaction, and gene expression profiling have also added important information to the more sophisticated sub grouping of AML.⁵ With optimal application of these techniques in the diagnosis of acute leukemias, the treatment strategies can be more specifically directed and

new therapeutic approaches can be evaluated more effectively.¹⁰

Keeping in view the diagnostic and prognostic significance of cytogenetic abnormalities, we report cytogenetic findings in 52 AML patients seen at our institution from January 2003 to January 2005, and to determine the impact of karyotype as a prognostic factor for response of induction chemotherapy.

Patients and Methods

A cross sectional study was carried out at the department of Pathology and Oncology, Aga Khan University Karachi (AKUH).

Consecutive patients admitted to the hospital with all of the following criteria were included in the study:

(a) Diagnosis of AML made between January 2003 to January 2005; (b) all age groups; (c) absence of prior history of malignant disease, cytotoxic or radiation therapy, myelodysplasia and (d) Submission of bone marrow sample for cytogenetic analysis before initiation of therapy.

Patients were treated according to standard protocol of induction chemotherapy with cytosine arabinoside for 7 days and anthracycline for 3 days. Seven patients with M3 FAB subtype received all-trans retinoic acid (ATRA) in conjunction with chemotherapy for induction of remission. Remission status was checked after 4 weeks of induction chemotherapy. Informed consent was obtained from patients or parents as appropriate.

The diagnosis of AML was based on morphologic and cytochemical studies of peripheral blood smears and bone marrow aspirate obtained before therapy was initiated. FAB criteria were used for classification and subclassification of the disorder⁵ and reviewed by two independent observers who lacked knowledge of cytogenetics results. All the peripheral blood and bone marrow films were stained with Leishman's stain. Additionally following cytochemical stains were used: periodic acid-Schiff (PAS) reagent, Myeloperoxidase (MPO), Sudan Black B (SBB) and a-naphthyl acetate esterase (ANAE). Immunophenotyping was done in some cases where considered essential. Haematological parameters were obtained by Coulter Cell Counter.

Cytogenetic analysis was performed using a Trypsin-Giemsa banding technique.¹¹ Cells were obtained from aspirated bone marrow before therapy was initiated. Metaphase cells were examined from direct preparations and/ or short-term (24-, 48-, and 72-h) unstimulated cultures. Whenever possible, at least 20 mitosis were analyzed. Karyotypes were interpreted using International System for Cytogenetic Nomenclature (1995) criteria.¹²

Cytogenetic abnormalities were grouped according to published criteria adopted by Southwest Oncology Group (SWOG) into favourable, intermediate and unfavourable risk categories.¹¹

Bone marrow was performed after 4 weeks of induction chemotherapy to determine the remission status. Complete remission (CR) was defined either as a normocellular bone marrow aspirate containing less than 5% blast cells and absence of Auer rods with evidence of normal maturation of other marrow elements or the bone marrow biopsy with more than 20% cellularity and maturation of all cell lines having less than 5% blast cells. Full recovery of normal peripheral blood count is not required to define CR.^{2,13} Remission failures were classified as deaths due to induction chemotherapy within 30 days or persistent disease showing more than 5% blast cells in bone marrow at the end of 30 days.

The data was analyzed using SPSS software (version 12.0.1). The following variables were studied for their prognostic value on the achievement for CR: age, sex, haemoglobin level, white cell count, platelet count and karyotype. Mann-Whitney U test and Chi-Square test were used to see the association between variables.

Results

A total of 56 patients were seen, 4 patients having inadequate samples for complete cytogenetic analysis were excluded from study. Out of 52 patients that were studied, 32 were males and 20 were females with a male to female ratio of 1.6:1. Their ages ranged between 9 months to 73 years. The mean haemoglobin concentration was 8.6g/dl (range 3.4-12.4), mean white cell count was $37.3 \times 10^9/L$ (range 1.3-168.3), and mean platelet count was $34.3 \times 10^9/L$ (range 1-180). The frequency of various FAB types is shown in Figure.

Of the 52 patients with adequate samples, 35 (67.3%) had a normal karyotype, and 17 (32.7%) had

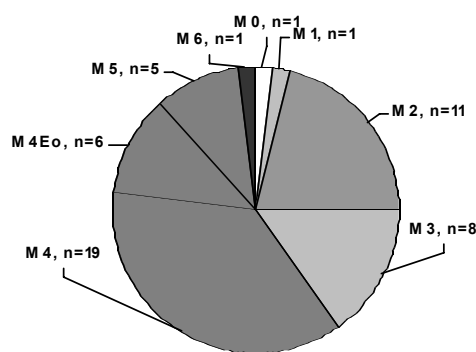


Figure. Frequency of FAB types in AML patients (n = 52)

cytogenetic abnormalities. Findings of cytogenetic results and their association with various FAB sub groups are shown in Table 1.

Table 1. Cytogenetic results and their association with FAB sub-groups in AML Patients (n = 52) and Complete remission results by cytogenetic risk group AML patient (n=41).

Cytogenetic results and association with FAB subgroups in AML patients (n = 52)		
Number of Patients	Cytogenetic results	FAB Subgroups
35	Normal Karyotype	M0,M1,M2,M3,M4, M4Eo,M5,M6, M2,M4, M4Eo
4	Hyperdiploidy	M2
4	45,X,-Y,t(8;21)(q22;q22). 45,X,-Y,t(8;21)(q22;q22). 46,XY,del(9)(p13;p24). 45,X,-X,t(8;21)(q22;q22) t(11;17)(q23; q25)?-13q.	M2
2	46,XX,?del(6)(q13;q14). 46,XY,t(15;17)(q22;q21).	M3
5	47,XX,+19. Trisomy 8. Trisomy 8. 45,XY,-7,del(11)(q23). 46,XX,t(6;11)(25;q22).	M4
1	47,XX,+12.	M4Eo
1	49,XY,t(2;15)(q37;q21), t(7;9)(q15;q34),+8,+10,+19.	M5
Complete remission results by cytogenetic risk group AML patient (n=41)		
Risk group	No. of Patients	No. (%) achieving CR
Favourable	3	3(100%)
45,X,-Y,t(8;21)(q22;q22). 45,X,-Y,t(8;21)(q22;q22) t(11;17) (q23;q25),?-13q. 46,XY,t(15;17)(q22; q21).		
Intermediate	32	15 (46.9%)
Normal. + 8. hyperdiploidy. 46,XX,?del(6)(q13;q14).		
Unfavourable	6	3 (50%)
47,XX,+19. 45,XY,-7,del(11)(q23). 49,XY,t(2,15)(q37;q21),t(7;9) (q15;q34)+8,+10,+19. 47,XX,+12. 46,XX,t(6;11)(q25;q22). 46,XY,del(9)(p13;p24).		

Eleven patients did not receive treatment either because of their death even before the start of chemotherapy or due to other reasons. Among the 41 treated patients, 21 (51.2%) achieved CR with induction chemotherapy. According to cytogenetics, the favourable risk group 3/3 (100%) achieved complete remission (CR) while the same was achieved by 15/32 (46.9%) in intermediate risk group and 3/6 (50%) in unfavourable risk group Table 1.

Prognostic value of other initial parameters on the achievement of CR was also analyzed by univariate analysis. Results are shown in Table 2. Significant lower CR was found in patients with a high white cell count whereas other factors revealed no prognostic value in our study.

Table 2. Prognostic factors associated with achievement of CR in AML patients (n=41).

Parameter	Mean Rank	P value
Age (years)		
CR	20.19	0.665
No CR	21.85	
Sex +		
CR		0.536
No CR		
Haemoglobin (g/dl)		
CR	20.57	0.821
No CR	21.45	
White cell count 109/L		
CR	15.48	0.002
No CR	26.80	
Platelet count 109/L		
CR	21.79	0.675
No CR	20.18	

+ X2 - test applied

Discussion

The importance of cytogenetic studies in neoplasia in general and in leukaemias in particular has been universally accepted. For the last two decades, it has been appreciated that diagnostic cytogenetics provide one of the most valuable prognostic indicators in AML.¹⁴⁻¹⁵ However, many studies on which such conclusions drawn were compromised to a variable extent either by relatively small sample size or by inconsistency of treatment approach. These limitations resulted in undermining the employment of karyotype at diagnosis. Unfortunately our study also has a relatively small sample size.

In the present study, abnormal karyotype was seen in 32.7% patients with denovo AML. Most studies reported an abnormal karyotype between 54-78% of patients^{16,17} although both lower¹⁸ and higher¹⁹ percentages have been reported. A study by Marcucci et al²⁰ revealed normal karyotype in 45% adult denovo AML patients. Similar results were drawn by Estey et al²¹ with the identical karyotypes at diagnosis and relapse. So a stable karyotype was most frequent among patients who presented without cytogenetic abnormalities, suggesting that normal karyotype is usually not due to sampling error. But in our study, it was not possible to do cytogenetic analysis in patients with normal karyotype presenting with relapse or not in remission due to financial constraints. We found t(8;21) in 3 cases of M2 and t(15;17) in one case of M3 subtypes.

But t(16;16) or inv (16) was not found in any case. The yield is low when compared with international studies. The reason for these findings is not known, probably due to inexperience particularly in the recognition of subtle structural aberrations that can sometimes be overlooked, particularly in preparation of sub-optimal quality. A study by Grimwade et al²² reported 7 cases of APML lacking t(15;17) on conventional cytogenetic assessment. In 6 of 7 cases, cryptic PML-RARa rearrangements were identified by reverse transcriptase polymerase chain reaction (RT-PCR) and fluorescent insitu hybridization (FISH). In the remaining one case variant translocation, t(11;17) was identified. Frohling et al²³ detected inv (16)/t(16;16) in 4 cases using FISH. Veldman et al²⁴ used multicolour spectral karyotype (SKY) in 15 cases with unidentified chromosome aberrations. So molecular cytogenetics should also be considered in cases with insufficient yields of metaphase cells, poor chromosome morphology and normal karyotype.

Most studies reported over all response to induction chemotherapy (expressed by the CR rate) between 70 to 80% using conventional protocol with cytarabine and daunorubicin or cytarabine and escalated doses of daunorubicin and etoposide.^{11,18,25} Our study has comparatively a lower rate of overall response to induction chemotherapy which is probably due to small number of patients or delayed referral of patients to a tertiary care centre. Various international studies reported 80-90% CR in favourable groups^{2,11,15} while 70-80% in the intermediate group and 50-60% in the unfavourable group.^{2,11} However a study by Pelloso et al.¹⁵ showed 20% and 12.5% CR in the intermediate and the unfavourable groups respectively. Our results in the favourable and unfavourable groups are in accordance with these studies.^{2,11} However, our intermediate group showed lower remission rate compared to some studies^{2,11} but the results were better, than that reported by Pelloso et al.¹⁵ The differences in CR in various risks groups from

present study is partly due to small number of patients in various groups or it may be due to improved and intensive chemotherapy protocols and good supportive care.

However, in the present study, favourable risk group showed higher⁷ remission rates² when compared to intermediate and unfavourable risk groups. This clearly establishes diagnostic karyotype as one of the most important prognostic factor for outcome of treatment in patients with AML. In patients with normal karyotype on conventional cytogenetics, sophisticated techniques should be applied to find subtle changes. A large number of patients with long term follow up and multicenter studies are needed for comparison of cytogenetic results and outcome of treatment with international studies.

Acknowledgement

The authors thank Mr. Nasheed Irshad and Munir Gillani for typing of manuscript, Mr. Salman Sabir for statistical analysis and Dr. Bushra Moiz for review of the manuscript.

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