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Correlation between Additional Cytogenetic Abnormalities and Clinical Outcome in Chronic Myeloid Leukemia

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Authors' contributions

This work was carried out in collaboration between both authors. Author PS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author DKG managed the analyses of the study. Both authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Introduction: Chronic myeloid leukaemia (CML) is a clonal myeloproliferative disorder characterized by massive proliferation of myeloid cell line with normal differentiation. The focus of mechanism is a balanced translocation t(9;22)(q34;q11.2) resulting in BCR-ABL1 oncogene. Additional cytogenetic abnormalities (ACAs) have been observed during transformation of chronic myeloid leukaemia from chronic to terminal phases is supposed to play a pivotal role in disease progression.

Materials and Methods: We conducted a prospective observational study including 100 patients of newly diagnosed Philadelphia positive chronic myeloid leukaemia. Patients were followed up for 12 months.

Results: The prevalence of additional cytogenetic abnormalities was 8 percent. Additional cytogenetic abnormalities positivity was associated with an increased mean value of Sokal score when compared to additional cytogenetic abnormality negative patients. At the end of 12 months, 62.50% of additional cytogenetic abnormalities positive patients showed progression to a more advanced phase compared to 7.61% in the additional cytogenetic abnormalities negative subgroup. 77.17 percent of additional cytogenetic abnormalities negative patients and 25 percent of additional cytogenetic abnormalities positive patients were in remission at the end of 12 months.

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Summary: Presence of additional cytogenetic abnormalities at initial diagnosis of chronic myeloid leukemia is associated with a higher risk of progression to an advanced phase. Early identification of these abnormalities may help in designing a more appropriate therapeutic approach.

Keywords: Cytogenetic abnormalities; chronic myeloid leukemia; clonal myeloproliferative.

ABBREVIATIONS

CML : Chronic myeloid leukemia
ACA : Additional cytogenetic abnormalities
Ph chromosome : Philadelphia Chromosome
CP : Chronic phase
AP : Accelerated phase
TKI : Tyrosine kinase inhibitors

1. INTRODUCTION

Chronic myeloid leukemia is a clonal myeloproliferative disorder characterized by massive proliferation and accumulation of myeloid line of cells that differentiate normally [1,2]. CML accounts for approximately 15 percent of all cases of leukemia. There is a slight male preponderance with a male: female ratio of 1.9:1.1. The focus of mechanism involves a reciprocal balanced translocation between the long arms of chromosomes 9 and 22. The derivative chromosome resulting from this reciprocal translocation is known as the Philadelphia chromosome [3]. This translocation results in an abnormal gene product, the BCR-ABL1 oncogene, coding for a constitutively active tyrosine kinase, leading to excessive proliferation and reduced apoptosis of CML cells [4,5]. These clonal cells have a growth advantage over their normal counterparts and thus resulting in suppression of normal haematopoiesis as the disease progresses.

The disease course can be divided into three phases namely the chronic phase, accelerated phase and a terminal blast phase. The use of tyrosine kinase inhibitors in treatment of CML has changed the natural history and prognosis dramatically. Before the introduction of TKIs, the median survival in CML was 3-7 years, with the 10-year survival rate being 30 percent or less. With the use of imatinib, the first approved TKI, today the 10-year survival rate has gone up to 83.3 percent [6].

Untreated, the disease is characterized by the inevitable transition from a chronic phase to an accelerated phase and on to blast crisis in a median time of 4 years [7,8]. The latter is an acute leukemia, and is further characterized by a block in differentiation. The vast majority of patients present in the chronic phase, many as an incidental finding on a full blood count. Common presenting features include fatigue, night sweats, weight loss, mild anaemia and symptoms relating to splenomegaly resulting from extramedullary haematopoiesis. Less common presenting complaints include hyper-viscosity related events such as thrombosis, myocardial infarction, visual disturbances, priapism and cerebrovascular accidents. Bleeding diathesis may occur resulting in retinal haemorrhage and gastrointestinal bleeding. A minority of patients are diagnosed with advanced phases of the disease without any history of antecedent chronic phase. Symptoms here relate to more severe anaemia, thrombocytopenia and splenomegaly. Additional symptoms such as unexplained fever, severe fatigue, joint pain, bleeding, thrombotic events and infections are more common in those patients presenting in advanced phases.

The mechanism behind the transformation of CML from chronic to terminal phases, are poorly understood. Association of additional cytogenetic abnormalities have frequently been observed, resulting in molecular events which finally culminates in genetic instability [3]. The frequency of additional cytogenetic abnormalities increases with advancing stage of CML, and is reported in about 30 percent of cases with accelerated phase and up to 80 percent in blast phase [7,8].

The clinical and prognostic significance of these additional cytogenetic abnormalities have not been well described. However, they have been found to be associated with a higher haematological relapse rate, lower complete cytogenetic remission rate and shorter overall survival [7]. Regardless of the underlying mechanism, the net result of additional cytogenetic abnormalities is the potential for a more malignant phenotype and possibly, less dependence on BCR-ABL1 for proliferation and survival [3,7], accounting for resistance and treatment failure.

A study conducted in 2016 to investigate the impact of additional cytogenetic abnormalities in Philadelphia-positive clone has shown that the Ph positive ACA emergence at diagnosis and during treatment had negative impact on overall prognosis of CML patients treated with TKIs [9].

Another study conducted in 2010 to investigate the impact of additional chromosomal aberrations and BCR-ABL1 kinase domain mutations on the response to nilotinib in Ph positive CML has shown that the presence of additional chromosomal aberrations may reflect genetic instability and, therefore, intrinsic aggressiveness of the disease which will be less amenable to subsequent alternative treatments [10]. In the study, patients with ACA had significantly worse overall survival (54%) than patients without ACA (89%, *P*=0.0025) [10].

Two large studies have confirmed the prognostic impact of ACAs detection. An Italian Working Group study which evaluated 378 patients, detected the presence of ACAs in 21 (5.6%) patients for whom the time to achieve complete cytogenetic response and major molecular response were longer [11]. Long-term results in patients with ACAs were inferior but the differences were not significant compared to standard CML patients [11]. A German working group study identified the presence of ACAs in 79 (6.9%) of 1151 enrolled and Imatinib treated patients [12]. For those patients, the time to achieve complete cytogenetic response and molecular remission was longer. Both progression free survival and overall survival were shorter in patients with ACAs at initial diagnosis than in patients with standard translocation (9; 22).

Thus, the detection of additional cytogenetic abnormalities in addition to Ph chromosome, in CML patients, may prove to have a vital role in treatment protocols currently employed. Classification of patients in various prognostic groups would enable a closer monitoring and consideration of other therapeutic strategies or a dose increase of imatinib. Our objective in this study is to evaluate the impact of these additional cytogenetic abnormalities on the clinical and haematologic profile of the patient and their overall impact on the prognosis of the patient.

2. MATERIALS AND METHODS

Set up: Study was conducted at V.M.M.C and Safdarjung hospital, at department of medicine and department of haematology.

Sample Size: 100

Study Design: Observational Prospective Study.

Inclusion criteria: Newly diagnosed patients of CML, with Philadelphia chromosome positive, within the age group of 12 to 80 years were included in the study.

Exclusion criteria: Pregnant patients.

2.1 Methodology

Based on the inclusion and exclusion criteria, newly diagnosed cases of CML were taken with informed consent and evaluated with detailed history including demographics, family, medical and past history and clinical examination.

In the first part of the study, complete blood counts with peripheral smear were done followed by bone marrow biopsy and cytogenetic analysis using G- banding technique.

G- banding: Giemsa banding (G- banding) technique was used to stain condensed chromosomes to produce a photographic representation of the chromosome sets. Cells derived from the specimen were cultured and dividing cells were arrested in metaphase via addition of colchicine. The cells were then treated with hypotonic saline solution which results in lysis and spreading of chromosomes. Fixation was done with a freshly prepared fixative (3 parts of methanol and 1 part of glacial acid). The metaphase chromosomes were treated with trypsin to partially digest the chromosome and relax the chromatin structure. The condensed chromosomes were stained with Giemsa stain. The individual chromosomes were arranged in a standardized format. After staining the bands can be studied under a light microscope. The light and dark bands on the chromosomes produced due to euchromatin and heterochromatin respectively was used for identification of the chromosome by their unique banding patterns. Around 15-20 metaphase spreads were analysed to reduce the likelihood of technical artefacts.

Patients were classified into chronic phase, accelerated phase and blast phase as per ELN guidelines (see appendix) and the prevalence of ACAs in each phase was calculated. Sokal score was calculated according to the data available at the initial presentation and the patients were classified into low risk, intermediate risk and high-risk groups.

In the second part of the study, patients were followed up at 3 months, 6 months and 12 months respectively. Prognostic significance was assessed in terms of progression, clonal evolution, cytogenetic response and the overall survival of the patients.

2.2 Statistical Analysis

Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean ± SD and median. Normality of data was tested by Kolmogorov-Smirnov test. If the normality was rejected then non parametric test was used.

Statistical tests were applied as follows-

1. Quantitative variables were compared using Mann-Whitney Test (as the data sets were not normally distributed) between the two groups.
2. Qualitative variables were correlated using Chi-Square test/Fisher's Exact test.

A p value of <0.05 was considered statistically significant.

The data was entered in MS EXCEL spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0.

3. RESULTS

Age distribution: The prevalence of CML was highest in the second and third decades, with 37 percent of patients between 15 to 30 years and 35 percent of patients in 31 to 40 years. The prevalence decreased thereafter with 23 percent of patients in the fifth decade and only 5 percent in sixth decade. Majority of ACA positive CML patients were found to be between 31 to 40 years of age. In contrast, only 32.61 percent of ACA negative patients were in this age range. The *P*-value was calculated to be 0.007 which was significant.

Sex: No significant variation with gender was observed in CML patients. 53 percent of patients were females and 47 percent were males. ACA positivity was equal in both sexes. No correlation was observed.

Phase of Presentation: Majority of patients presented in chronic phase from both ACA positive and ACA negative subgroups. However, 25 percent of ACA positive patients were in accelerated phase at initial presentation, in comparison to 5.43 percent in ACA negative patients. 1 percent of ACA positive patients were in blast crisis while none of ACA negative patients were in blast crisis at initial presentation. The difference between the ACA positive and negative groups was significant (p-value 0.0003), with ACA positive patients having a tendency to present in a more advanced stage.

Outcome at 3 Months: At the end of 3 months follow up, disease course did not vary significantly between both subgroups. Progression was observed in 12.50 percent of ACA positive patients and in 3.26 percent of ACA negative patients. The P-value was 0.425 which was not significant.

Outcome at 6 Months: At the end of 6 months follow up, there was a significant difference between the disease course among ACA positive and ACA negative patients. 62.50 percent of ACA positive patients showed progression, compared to 6.52 percent of ACA negative patients. The p-value was <0.0001, which was significant.

Outcome at 12 Months: At the end of 12 months of follow up, the difference in disease course among ACA positive and ACA negative patients was maintained. 62.50 percent of ACA positive patients had progression in stage of disease in comparison to 7.61 percent of ACA negative patients. The p-value was significant at <0.0001.

Correlation of ACAs with Clonal Evolution: Clonal evolution was observed in 37.50 percent of ACA positive patients and 4.35 percent of ACA negative patients. The difference was significant with a p-value of 0.010.

Table 1. Phase at initial presentation

		ACA		Total	P value
		Absent	**Present**		
Phase	Chronic Phase	87 (94.57%)	5 (62.50%)	92 (92.00%)	0.0003
	Accelerated Phase	5 (5.43%)	2 (25.00%)	7 (7.00%)	
	Blast Crisis	0 (0.00%)	1 (12.50%)	1 (1.00%)	
Total		**92 (100.00%)**	**8 (100.00%)**	**100 (100.00%)**	

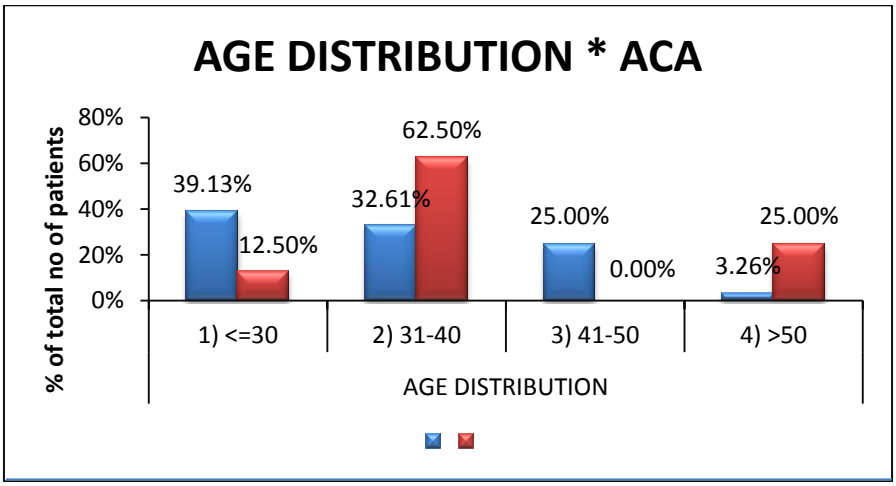

Fig. 1. Age distribution in relation to ACAs

Table 2. Patient outcome during follow up

	3 months		6 months		12 months	
	ACA absent	**ACA present**	**ACA absent**	**ACA present**	**ACA absent**	**ACA present**
No progression	88 (95.65%)	7 (87.50%)	78 (84.78%)	2 (25.00%)	73 (79.35%)	2 (25.00%)
Progression	3 (3.26%)	1 (12.50%)	6 (6.52%)	5 (62.50%)	7 (7.61%)	5 (62.50%)
Lost follow up	1 (1.09%)	0 (0.00%)	8 (8.70%)	1 (12.50%)	12 (13.04%)	1(12.50%)
P value	0.425		< .0001		< .0001	

Table 3. Clonal evolution

		ACA		Total	P value
		Absent	**Present**		
Clonal Evolution	Absent	88 (95.65%)	5 (62.50%)	93 (93.00%)	0.010
	Present	4 (4.35%)	3 (37.50%)	7 (7.00%)	
Total		**92 (100.00%)**	**8 (100.00%)**	**100 (100.00%)**	

Table 4. Endpoint outcome

		ACA		Total	P value
		Absent	**Present**		
Endpoint	No progression	71 (77.17%)	2 (25.00%)	73 (73.00%)	<.0001
	Progression	7 (7.61%)	5 (62.50%)	12 (12.00%)	
	Lost follow up	13 (14.13%)	1 (12.50%)	14 (14.00%)	
	Expired	1 (1.09%)	0 (0.00%)	1 (1.00%)	
Total		**92 (100.00%)**	**8 (100.00%)**	**100 (100.00%)**	

Table 5. Types of ACA and risk category

S. no.	ACA type	Sokal score	Risk category
1.	Inv. 17	3.3	High risk
2.	Inv. 11	1.1	Intermediate risk
3.	t(8;17), t(6;11), t(2;7), q(21;22)	1.5	Intermediate risk
4.	t(2;16)	2.0	High risk
5.	t(13;22)	1.2	Intermediate risk
6.	t(5;7)	1.0	Intermediate risk
7.	Trisomy 8	1.1	Intermediate risk
8.	Hypoploidy 42-44	1.2	Intermediate risk

Table 6. Correlation of ACA with Sokal score

	ACA		P value
	Absent	**Present**	
Age			0.141
Sample size	92	8	
Mean ± SD	35.39 ± 9.72	42.38 ± 12.72	
Median	35	39	
Min-Max	17-60	28-62	
Inter quartile Range	28 - 42.500	34.500 - 51	
Sokal			0.001
Sample size	92	8	
Mean ± SD	1.01 ± 0.8	1.55 ± 0.78	
Median	0.9	1.2	
Min-Max	0.6-7.1	1-3.3	
Inter quartile Range	0.700 - 1	1.100 - 1.750	

Effect of ACAs on Endpoint: ACA positive patients were observed to have lower rates of remission and significantly higher rates of progression to an advanced stage. 77.17 percent of ACA negative patients were in remission at the end of 12 months in contrast to 25 percent of ACA negative patients.

ACA Association with Risk Category: ACA positivity was correlated with a higher Sokal Score at initial presentation. Out of the 8 patients who were positive for ACAs, 2 patients were in high risk category and 6 were in intermediate risk category.

Correlation of ACAs with Sokal Score: ACA positivity was correlated with a higher Sokal Sore. The mean Sokal Score in ACA positive patients was calculated to be 1.55 with a standard deviation of 0.78. In ACA negative patients the mean Sokal Score was 1.01 with a standard deviation of 0.8. Median values for Sokal Score was 0.9 for ACA negative patients and 1.2 for ACA positive patients. The difference between both subgroups was significant with a p-value of 0.001.

4. DISCUSSION

The study included a total of 100 patients of newly diagnosed CML who were positive for Philadelphia chromosome.

Majority of the CML patients were found to be in the age range of 15 to 40 years (72 percent). This finding varies from existing literature where the median age of diagnosis of CML has been established between 45 to 55 years. Compared to existing data, our patients demonstrated a trend towards an earlier age of onset and presentation. At the time of presentation, 92 percent of the patients were in the chronic phase of disease, 7 percent in accelerated phase and one percent presented in blast crisis. The prevalence of ACAs at initial presentation was found to be 8 percent. This was in consistency with findings from previous studies where the prevalence of ACAs was found to be between 5 to 10 percent [7]. At the end of the follow up period, 73 patients had no progression of disease, 12 patients showed progression, 14 patients were lost to follow up and 1 patient expired during the follow up period.

The presence of ACAs showed a predominance in the age group of 31 to 40 years, with 65 percent of patients with ACAs falling in this age range. Previous studies have not reported any age selectivity for the presence of ACAs in CML.

Out of total 100 patients of CML included in this study, 53 patients were female and 47 were male. The slight female preponderance observed could not be compared to existing data as the incidence was not age adjusted.

There was no gender predilection for the prevalence of ACAs and there was an equal distribution in male and female patients. Previous studies have not demonstrated any gender bias regarding the presence of ACAs.

ACA positivity at the initial presentation was found to be associated with an increased mean value of Sokal score. From the ACA positive subgroup, the mean Sokal score was calculated to be 1.55 with standard deviation of 0.78. On comparison to ACA negative subgroup, the mean Sokal score was 1.01 with a standard deviation of 0.8 in these patients. This difference was

found to be significant with a p-value of 0.001. There are no previous studies in this area where the relationship between ACAs and the initial clinical scoring system has been established. This study demonstrates the association of ACAs with a higher Sokal Score at initial presentation.

Majority of the ACA negative patients presented in the indolent phase of the disease. 87 out of 92 patients (94.57 percent) who were ACA negative, were in the chronic phase, and 5 patients (5.43 percent) were in accelerated phase. In contrast, ACA positivity was associated with a more aggressive presentation. Of the 8 patients who were ACA positive, 5 patients (62.50 percent) were in chronic phase, 2 patients (25.00 percent) were in accelerated phase and 1 patient (12.50 percent) was in blast crisis. The difference was statistically significant with a p-value of 0.0003. A previous study has demonstrated a similar finding where the presence of ACAs was significantly associated with accelerated phase [13].

At the end of 3 months of follow up period, there was no statistically significant difference in the disease course between ACA positive and ACA negative patients. 95.65 percent of the ACA negative subgroup and 87.50 percent of ACA positive subgroup did not show progression of disease. 3.26 percent of ACA negative patients showed progression whereas 12.50 percent of ACA positive patients had progression of disease.

At 6 months of follow up, a significant difference was observed among both subgroups with ACA positive patients having a more aggressive change in the disease course. 84.78 percent of ACA negative patients did not have any disease progression, whereas 6.52 percent had progression. From the ACA positive subgroup, 62.50 percent of patients had progression of disease while 25.00 percent did not have any progression. The difference was highly significant with a p-value of less than 0.0001.

At the end of 12 months of follow up, 79.35 percent of patients from ACA negative subgroup did not have any progression of disease while 7.61 percent showed progression. Comparing these values with ACA positive subgroup, it was observed that 62.50 percent of ACA positive patients showed progression. Again, this difference was statistically significant with a p-value of less than 0.0001.

Previous studies have demonstrated the association of ACAs with a worse outcome in terms of cytogenetic response, progression free interval and overall survival. The above findings from this study shows the presence of ACAs to be associated with a more malignant disease course with higher rates of disease progression compared to ACA negative CML patients. Progression free interval was significantly shorter in ACA positive patients compared to ACA negative subgroup.

During follow up, clonal evolution was observed in 7 percent of patients. This finding was consistent with a previous study where the overall incidence of clonal evolution was found to be 8 percent after a median follow up of 30 months [14]. In this study, 4.35 percent of ACA negative patients and 37.50 percent of ACA positive patients showed clonal evolution. The difference was not significant (p-value 0.010). Thus, it is not clear whether the presence of ACAs at diagnosis increases the incidence of clonal evolution.

At the end of 12 months follow up, the presence of ACAs was found to have an adverse impact on disease course with 62.50 percent of ACA positive patients showing progression in contrast to 7.61 percent of ACA negative patients who had progression. 77.17 percent of ACA negative patients and 25.00 percent of ACA positive patients had no progression at the end of 12 months. The difference was highly significant with a p-value of less than 0.0001. The findings from this study reaffirms the adverse impact of ACAs on the disease course of CML.

5. CONCLUSION

In this study, the prevalence of additional cytogenetic abnormalities in patients of chronic myeloid leukemia was found to be 8 percent in the studied population. Majority of the patients with ACAs were in the age range of 31 to 40 years. There was no gender association in the prevalence of ACAs. The presence of ACAs was associated with high risk disease as defined by increased Sokal scores. The mean Sokal score in ACA positive CML patients was 1.55 (with standard deviation 0.78) in contrast to ACA negative CML patients who had a mean Sokal score of 1.01 (standard deviation 0.8).

The presence of ACAs was also associated with a more advanced initial presentation and aggressive disease course. The majority of ACA

positive CML patients showed disease progression during the 12 months follow up period and decreased rates of cytogenetic remission. The impact on overall survival did not appear to be changed but this may be due to short follow up period and patients being lost to follow up. The study subgroups did not show any significant difference in presence of clonal evolution between ACA positive and ACA negative CML patients.

One of the strengths of this study was the correlation of ACAs with the Sokal Score at diagnosis. Previous studies have been lacking in demonstrating a relationship between ACAs and the initial clinical scoring. Another point of note was the correlation of ACAs with clonal evolution which has not been reported from previous studies. Also there is a paucity of data from the Indian population regarding the association of ACAs with progression of CML and this study aims to provide a better insight about the impact of ACAs on clinical and haematological profile of CML in Indian patients.

Limitations of the study included inability to assess response to tyrosine kinase inhibitor treatment as treatment adherence could not be evaluated. Also, the true impact on long term prognosis of ACA positive CML patients could not be commented upon due to limited follow up period. Further studies in this area are required to assess the impact of ACAs upon response to tyrosine kinase inhibitors and long-term prognostic significance of ACAs.

CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

ELN classification of CML:

Chronic phase:

1. Presence of specific translocation [t(9;22) (q34;q11.2)] and derivative chromosome (ph chromosome).
2. Not fulfilling the criteria for accelerated phase and blast phase.

Accelerated phase (any 1 or more criteria):

I. Blasts 10-19% in peripheral blood or bone marrow.
II. Peripheral blood basophilia at least 20%.
III. Persistent thrombocytopenia (< 100 × 10^9/l) unrelated to therapy or persistent thrombocytosis (>1000 × 10^9/l) unresponsive to therapy.

Blast phase (any 1 or more criteria):

I. Blasts ≥20% of peripheral blood white cells or bone marrow cells.
II. Extra medullary blast proliferation.
III. Large foci or clusters of blasts in bone marrow biopsy.

Definition of clonal evolution: The development of ACAs in a proportion of ph positive cells during the course of disease.

Table 1. Risk stratification and prognostic scoring: Sokal Score

S.no.	Parameter	Score
1.	Age	0.0116 (age – 43.4 years)
2.	Spleen size in (cm below costal margin)	0.0345 (spleen size – 7.51)
3.	Percent of blasts in peripheral smear	0.0887 (blasts – 2.1)
4.	Platelet count x 10^9	0.188 [(platelets/700)^2 – 0.563]

Table 2. Calculation and stratification

Relative risk	Score = exponential of total of above scores
Low risk	<0.8
Intermediate risk	0.8 – 1.2
High risk	>1.2

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