Article published online: 2025-07-16

Chronic Myelogenous Leukemia Patients on Tyrosine Kinase Inhibitors—Hematological Changes and Correlation with European Leukemia Network Response Criteria

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Ind J Med Paediatr Oncol

Abstract

Introduction Chronic myeloid leukemia (CML) patients receive tyrosine kinase inhibitor (TKI) therapy for long duration. In this study, we analyzed hematological changes and compared them with hematological and cytogenetic response in 542 CML patients on TKI therapy with a follow-up period of 84 months.

Objective To study hematological changes in patients on TKI therapy.

Materials and Methods CML patients on TKI therapy (imatinib, 400 mg) with a minimum follow-up of 6 months were enrolled over a period of 8 years. Response was evaluated as per European Leukemia Network guidelines.

Results A total of 542 patients with CML were included in the study, with 507 (93.5%) being in chronic phase disease, 21 (4%) in accelerated phase, and 14 (2.5%) in blast crisis.

The median age of patients was 38 years (range: 14–77 years), with male:female ratio = 1.4:1 (males = 317, 58.5% and females = 225, 41.5%).

At 3 months, 90% patients achieved complete hematological response (CHR). Normalization of platelet count, total leucocyte count, marrow cellularity, and granulocytic hyperplasia occurred by 3rd month of TKI therapy in majority of patients. Even though platelet counts normalized by 3rd month, megakaryocytic hyperplasia in the marrow normalized by 12th month of TKI therapy only.

Cytopenias were invariably seen in all follow-up time points, with anemia and thrombocytopenia being most common. At 1 and 2 years, respectively, anemia was seen in 25 and 31% of patients, leucopenia in 3 and 1% patients, and thrombocytopenia in 7 and 4% patients. In addition, bicytopenia and pancytopenia, respectively, were seen in 14.5 and 3% patients at 1 year and in 9 and 4% patients at 2 years.

Marrow hypocellularity and lymphoid nodules were seen in nearly 20% patients during TKI therapy.

Keywords

- chronic myelogenous leukemia
- tyrosine kinase inhibitors
- hematological changes
- response

DOI https://doi.org/10.1055/s-0045-1810082.
ISSN 0971-5851.

Thieme Medical and Scientific Publishers Pvt. Ltd., A-12, 2nd Floor, Sector 2, Noida-201301 UP, India

Marrow hypercellularity was seen in a higher proportion of patients who were in "not in complete hematological response" (NCHR) than those who had achieved CHR (e.g., NCHR vs. CHR: at 6 months = 62 vs. 14%; at 12 months = 73 vs. 2%, at 18 months = 71 vs 9%, at 24 months = 57% vs. nil, at 36 months = 56 vs. 11%, at 42 months = 75 vs. nil, and at 60 months = 50% vs. nil).

None of the peripheral blood and bone marrow parameters analyzed in our study were consistently different between optimal and nonoptimal cytogenetic (warning and failure) responders.

Conclusion In this study, TKI-induced hematological changes were evaluated in CML patients.

The increased peripheral blood counts, marrow cellularity, and granulocytic hyperplasia seen at diagnosis normalized in 90% patients by 3rd month of TKI therapy.

In patients who achieved CHR versus NCHR, a higher proportion of NCHR patients showed bone marrow hypercellularity.

Between optimal and nonoptimal cytogenetic responders, none of the peripheral blood and bone marrow parameter was found to be significantly different.

During follow-up, TKI-induced hematological changes observed included cytopenia, marrow hypocellularity, and lymphoid nodules.

Introduction

Chronic myelogenous leukemia (CML) occurs due to a reciprocal translocation between chromosome 9 and chromosome 22, i.e., t(9;22)(q34;q11), leading to formation of Philadelphia (Ph) chromosome. This translocation results in fusion between Abelson murine leukemia viral oncogene homology 1 (ABL1) gene at chromosome 9 and break-point cluster (BCR) gene at chromosome 22. The chimeric *BCR::ABL1* oncogene encodes the BCR::ABL1 fusion protein with constitutional tyrosine kinase activity.[1]

Tyrosine kinase inhibitor (TKI) therapy is the primary modality of treatment for CML patients. TKI include imatinib mesylate (first TKI), dasatinib, nilotinib, bosutinib, and ponatinib. Prior to TKI discovery, the median survival of CML patients was 2 to 3 years, and the disease would transform from the chronic phase to accelerated or blast crisis phase. However, with TKI therapy, complete hematologic remissions in 97% and complete cytogenetic response (CCyR) in 86% of CML patients have been reported.[2]

However, TKI therapy, once started, has to be given for a long duration and the hematological (peripheral blood and bone marrow) changes associated with long-term TKI therapy have not been completely explored.

Peripheral blood changes reported with long-term use of TKI include cytopenias and relative lymphocytosis, and with dasatinib, in particular, raised large granular lymphocytes have been reported.[3–6] Bone marrow changes reported with treatment with imatinib include normalization of marrow cellularity, resolution of reticulin fibrosis, reduction in megakaryocyte number and clustering, relative erythroid hyperplasia, reactive lymphoid aggregates containing admixed T- and B-lymphocytes, increased new bone formation, prominence of pseudo-Gaucher cells, and reduction of sea-blue histiocytes. Myeloid hyperplasia associated with CML resolves mostly by 2 months, but megakaryocytic hyperplasia can take up to 5 months to resolve.[3] There can be normalization of bone marrow morphology, even in the absence of a cytogenetic response, indicating the effect of TKI therapy in blocking the action of BCR::ABL fusion protein.[3]

Some authors have additionally studied the hematological changes to predict the disease response.[7–9] Lugli et al proposed a morphological scoring system to evaluate the bone marrow changes associated with cytogenetic outcome. They found a significant correlation between CCyR with morphological parameters like normalization of bone marrow cellularity, absence of abnormal megakaryocytes, and reduction of marrow fibrosis.[7] Study by Srinivas et al followed up patients for 16 months and found a positive correlation between cytogenetic response and bone marrow changes, including normalization of bone marrow cellularity and myeloid hyperplasia.[8] Joshi et al had a shorter follow-up of 6 months for their patients, and did not find any significant correlation between cytogenetic response and bone marrow changes.[9] From these studies, it appears that morphological changes correlate with response, but a minimum of 12 month follow-up is required.

In the present study, with overall follow-up of 84 months, we have studied the hematological changes (peripheral blood and bone marrow) in CML patients on TKI therapy. Also, concurrent hematological, cytogenetic, and molecular responses with respect to the peripheral blood and bone marrow changes have also been compared.

Materials and Methods

Study Design

Descriptive.

Study Setting

Tertiary hospital–based.

The study analyzed retrospective data of 542 CML patients, diagnosed since 2008, over a duration of 8 years. All CML patients (age >14 years) on TKI therapy with a minimum of 6 months' follow-up were enrolled in the study. The median follow-up duration was 46 months (range: 3–96 months, from January 2008 to December 2015). The baseline and follow-up clinical data, peripheral blood and bone marrow findings, conventional cytogenetics for Ph chromosome, and reverse transcription-polymerase chain reaction (RT-PCR) for *BCR::ABL1* fusion gene were recorded. To ascertain nutritional cause of anemia, RBC cell indices and serum iron profile were recorded.

During follow-up, the responses were evaluated as per European Leukemia Network (ELN) recommendations.[10] Hematologic, cytogenetic, and molecular responses were assessed, and the treatment response was defined as optimal, suboptimal (warning), or failure (►**Supplementary Tables S1** and **S2, respectively [online only]).**

TKI therapy given included imatinib 400 mg daily. Other TKIs (dasatinib/nilotinib) were given if there was no response or side effects with imatinib were observed.

All patients in our cohort did not have regular bone marrow follow-ups at fixed time points, especially at 3rd month, but had uniform cytogenetic and/or RT-PCR monitoring. Due to this reason and for the ease of data interpretation and statistical analysis, the bone marrow and concurrent peripheral blood findings were analyzed at 6-month intervals for first 4 years (i.e., 6th, 12th, 18th, 24th, 30th, 36th, 42nd, and 48th months). After 4 years, subsequent data at 12-monthly intervals were recorded (60th, 72nd, and 84th months). In the 85th month to 96th month time interval, bone marrow follow-up data of only three patients were available, and hence were not included for morphologic assessment.

Inclusion and Exclusion Criteria

Inclusion: all adult CML patients (age > 14 years) diagnosed according to World Health Organization criteria on TKI therapy with a minimum of 6-month follow-up were enrolled.

Exclusion: (1) patients not willing to give consent and (2) patients not on TKI therapy.

Primary and Secondary Outcomes

Primary: status of peripheral blood counts and bone marrow status after start of TKI therapy.

Secondary: development of TKI resistance and development of kinase domain mutation due to TKI therapy.

Statistical Analysis

The statistics was calculated using MS EXCEL 2007 and SPSS (version 19) software.

- For comparing the mean between two groups samples: paired *t*-test and Wilcoxon signed-rank test (for continuous data) and Chi-squared test (for categorical data) were used.
- For comparing the mean between more than two groups: one-way ANOVA and Kruskal–Wallis test (for continuous data) and Chi-squared test (for categorical data) were used.
- All tests were two-tailed and were significant at $p < 0.05$.

Ethical Approval

The study was approved by Institutes Ethics Committee (No: INT/IEC/2015/182, dated June 08, 2015) and all procedures performed were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Results

During the study period, 1,371 patients were diagnosed as CML. Of these, 542 patients had a follow-up bone marrow evaluation done and these were included in the study for evaluating the peripheral blood and bone marrow changes occurring due to TKI therapy and for the comparison of hematologic, cytogenetic, and/or molecular responses with morphologic changes.

At the time of diagnosis, 93.5% ($n = 507$) of CML patients were in chronic phase, with 4% ($n = 21$) and 2.5% ($n = 14$) in accelerated and blast crisis phase, respectively. The clinical and hematological features at presentation are summarized in ►**Table 1**.

Spectrum of Peripheral Blood and Bone Marrow Changes in CML-CP Patients during TKI Therapy

Majority of our CML patients (94%) were in chronic phase during diagnosis. All these patients were started on daily dose of 400 mg imatinib mesylate. As the number of cases in accelerated and blast crisis groups was small, and to keep the study group uniform, the peripheral blood and bone marrow changes were further studied in chronic myeloid leukemia in chronic phase (CML-CP) patients only.

Peripheral Blood Changes during TKI Therapy

Complete Blood Counts during TKI Therapy

It was seen that hemoglobin, platelet count, and total leucocyte count (TLC) normalized in most of CML-CP patients at 3 months. ►**Table 2** provides details of peripheral blood changes observed in these patients over a follow-up period of 84 months and ►**Fig. 1** depicts the trend in hemoglobin, platelet count, and TLC.

Cytopenia during TKI Therapy

Patients without baseline cytopenias were evaluated to study TKI-induced cytopenias following therapy. During TKI therapy, cytopenias in the form of anemia (Hb < 13 g/L in males and <12 g/L in females), thrombocytopenia (platelet count $< 100 \times 10^9$/L), or leucopenia (TLC $< 4 \times 10^9$/L) were seen in isolation or in combinations.

Anemia was the most common cytopenia; however, the nutritional cause was the etiology in majority of cases. Leucopenia was not very common and was seen in 0 to 8% cases, at various follow-up time points. Thrombocytopenia was seen in 0 to 23% cases on follow-up at almost all time points. Therefore, thrombocytopenia was the most common TKI-induced cytopenia.

Table 1 Clinical and hematological parameters of CML patients at diagnosis

Parameter	CML phase			*p*-Value
	Chronic phase	Accelerated phase	Blast crisis	
Number	507	21	14	
Male:female ratio	1.4:1	1:1.3	1.8:1	0.308
Males	299 (55.2%)	9 (1.7%)	9 (1.7%)	
Females	208 (38.4%)	12 (2.2%)	5 (0.9%)	
Symptoms				
Incidentally detected	33 (6.5%)	0	0	0.297
Fever	148 (29%)	7 (33%)	6 (43%)	0.508
Abdominal heaviness	227 (45%)	14 (67%)	9 (64%)	0.055
Fatigue	203 (40%)	13 (62%)	9 (64%)	0.320
Loss of appetite	56 (11%)	3 (14%)	0	0.373
Loss of weight	37 (7%)	0	1 (7%)	0.439
Signs				
Palpable splenomegaly	364 (72%)	20 (95%)	13 (93%)	0.207
Median spleen size below left costal margin in cm	7 (0–30)	12 (0–22)	10 (0–24)	0.052
Palpable hepatomegaly	220 (43%)	13 (62%)	7 (50%)	0.224
Median liver size below right costal margin in cm	2 (0–2)	3 (0–16)	3 (0–10)	0.158
Lymphadenopathy	67 (13%)	2 (9.5%)	4 (28.5%)	0.218
Peripheral blood				
Hemoglobin, g/L	98 (35–166)	88 (43–110)	88 (44–140)	**0.012**
TLC, $\times 10^9$/L	173 (11–700)	156 (23–580)	100 (13–396)	0.135
Platelet count, $\times 10^9$/L	395 (10–1,300)	412 (45–1,058)	285 (7–1,006)	0.231
LAP score	28 (0–225)	39 (0–110)	54 (0–180)	0.096
Bone marrow				
Particulate aspirate	395 (78%)	9 (43%)	8 (57%)	**0.001**
Megakaryocyte				**< 0.001**
Increased	212 (42%)	6 (29%)	3 (21%)	
Adequate	253 (50%)	11 (52%)	5 (36%)	
Reduced	41 (8%)	4 (19%)	6 (43%)	
Dwarf megakaryocytes				0.227
≤10%	360 (71%)	4 (44%)	4 (80%)	
>10%	147 (29%)	5 (56%)	1 (20%)	
Reticulin fibrosis ($n = 233$)				
1+	50 (24%)	3 (21%)	0	**0.044**
2+	83 (39%)	1 (7%)	2 (28.5%)	
3+	66 (31%)	8 (57%)	4 (57%)	
4+	13 (6%)	2 (14%)	1 (14.5%)	

Abbreviations: CML, chronic myeloid leukemia; LAP, Leukocyte alkaline phosphatase; TLC, total leucocyte count.

Bone Marrow Changes during TKI Therapy

The follow-up, bone marrow was predominantly particulate with median myeloid to erythroid (M:E) ratio being 2:1 to 3:1, indicating that there was normalization of granulocytic and erythroid proportion of cells in the bone marrow during TKI therapy at all time points, starting from 3 months post-therapy time point itself.

Megakaryocytic hyperplasia was seen at 3rd and 6th months, at other time points predominantly there was normalization of megakaryocytes. Dwarf megakaryocytes

Table 2 Follow-up hemogram findings in CML-CP patients

Parameter	Months of TKI exposure											
	3	6	12	18	24	30	36	42	48	60	72	84
Hb, g/dL	92.5 (5.5–14.2)	11.2 (4.8–14.7)	11.2 (3.9–15.3)	11.3 (6.0–15.1)	11.5 (5.0–16.4)	11.0 (3.5–15.3)	11.0 (4.1–15.0)	11.0 (5.6–15.0)	10.5 (6.6–15.8)	10.5 (4.1–15.2)	12.0 (6.0–14.4)	11.9 (7.0–14.4)
PLT, $\times 10^9$/L	179 (14–660)	172 (5–1,005)	161 (4–1,387)	175 (5–1,132)	171 (4–1,455)	166 (3–1,139)	159 (8–1,411)	169 (23–1,050)	162 (31–348)	173 (13–1,849)	145 (50–255)	195 (114–312)
TLC, $\times 10^9$/L	4.2 (0.7–137)	6.8 (1.1–338)	5.9 (1.2–245)	6.1 (1.5–149)	6.7 (0.9–228)	6.3 (1.1–103)	6.1 (0.6–163)	6.4 (1.6–235)	5.8 (3–225)	6.3 (2–105)	5.9 (3.4–12)	6 (2.7–13)
LAP score	72 (28–207)	142 (4–362)	136 (4–397)	169 (0–378)	129 (6–380)	163 (7–344)	128 (0–380)	178 (2–322)	150 (5–328)	159 (5–360)	89 (23–294)	162 (45–274)

Abbreviations: CML-CP, chronic myeloid leukemia in chronic phase; LAP, Leukocyte alkaline phosphatase; PLT, platelet; TLC, total leucocyte count; TKI, tyrosine kinase inhibitor.
Note: For each parameter, the values are depicted in medians, with ranges in parenthesis.

(≥10%) could be seen in up to 20% cases at all follow-up time points.

The cellularity ranged from hypocellular to hypercellular.

Lymphoid nodules were also seen at all time points, in 0 to 33% cases.

The bone marrow aspirate and biopsy findings during long-term TKI therapy are elaborated in ►**Table 3**, and ►**Fig. 2** shows the compilation of bone marrow biopsy changes seen during TKI therapy.

Hematological Response Assessment and Disease Progression during Follow-Up in CML-CP Patients

At any given time point (ranging from 3 months to 84 months), majority of our patients (71–90%) were in complete hematological response (CHR), which was also reflected in the trend of hemoglobin, platelet count, and TLC during follow-up and also in the normalization of myeloid series of cells and megakaryocytes in the follow-up bone marrow.

However, despite majority of patients achieving CHR, progression to accelerated phase and blast crisis was also seen in 12 (2.3%) and 25 (4.9%) patients, respectively, during the follow-up period.

The details of CML-CP patients showing CHR or disease progression are outlined in ►**Table 4**.

Comparison of Peripheral Blood and Bone Marrow Changes in Patients on TKI Therapy—with Hematological Response (ELN Criteria)

Peripheral blood and bone marrow findings of patients in CHR versus NCHR (not in CHR) at follow-up time points are analyzed. During this analysis, patients who had progressed to accelerated phase or blast crisis were excluded at the respective time points of progression; as statistically significant differences in peripheral blood and bone marrow parameters are expected between the progressed and not progressed patients, therefore comparison will not add further information.

A summary of significant factors is as follows:

- At all time points, from 6th to 84th months, TLC, platelet counts, blast%, basophil%, and myelocyte% were significantly different between CHR and NCHR groups, these being higher in the NCHR group.
- Similarly, bone marrow cellularity and M:E ratio were also significantly different between the two groups, with NCHR patients showing hypercellularity.
- The leukocyte alkaline phosphatase (LAP) score was significantly different at 60th month between the CHR and NCHR groups, with patients in NCHR having lower LAP scores.

Comparison of Peripheral Blood and Bone Marrow Findings and Hematological Response—with Cytogenetic Response (ELN Criteria)

- At 6th month, the hematologic remission status in relation to cytogenetic response status was found to be statistically significant. Majority of patients with "optimal" (93%) cytogenetic response were also in

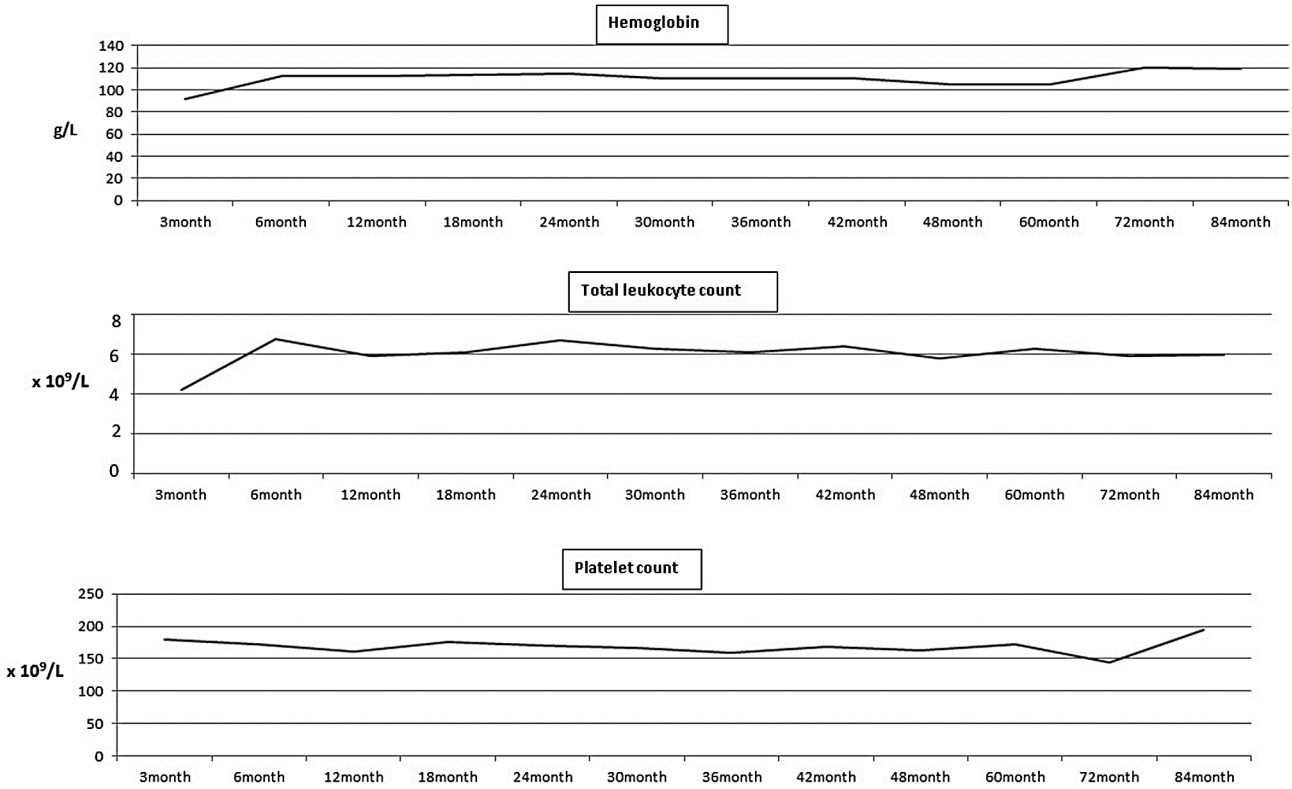

Fig. 1 Trend of median hemoglobin, platelet count, and total leukocyte count of CML-CP patients on follow-up. CML-CP, chronic myeloid leukemia in chronic phase.

hematological remission. However, nearly one-fourth (23%) of patients with "warning" were in NCHR and one-third (36%) with "failure" were in NCHR or showed an accelerated phase.

- At 24th month, the hematologic remission status in relation to cytogenetic response status was found to be statistically significant. Majority of patients with "optimal" (91%) cytogenetic response were also in hematologic remission.
- This correlation of hematologic remission status with the cytogenetic response status was not found to be statistically significant at 12th, 18th, 30th, and 36th month time points.
- None of the bone marrow parameters were found to be significantly different between the optimal and failure cytogenetic response groups.

►**Fig. 3** depicts hypercellular marrow spaces with megakaryocytic hyperplasia in a 49-year-old male who had lost hematologic remission and failed cytogenetic response at 24th month of TKI.

Analysis of Hematologic and Molecular Response with Respect to *BCR-ABL1* Transcript Type

The *BCR-ABL1* fusion transcript data (p210 or p190) were available for 334 patients. Of these 334 patients, p210 (e13a2 or e14a2) was seen in 333 patients (99.7%) and p190 (e1a2) transcript was seen in one case (0.3%) only. Among the p210 transcripts, e13a2 transcript was common, seen in 65% of patients.

At all the time points of follow-up, there was no significant difference in the hematologic, cytogenetic, or molecular response status between patients with either e13a2 or e14a2 fusion transcripts.

Discussion

CML is the most common myeloproliferative neoplasm. In India, CML accounts for 30 to 60% of adult leukemias.[11] The pathogenesis of CML is well-understood and this knowledge has been utilized in developing novel targeted therapies like TKIs. To maintain optimal response, these drugs have to be taken daily. The hematologic changes induced by long-term TKI therapy has not been well characterized over longer follow-up time points.

In the current study, we have retrospectively analyzed the hematologic changes induced by long-term TKI therapy in 507 CML-CP patients over a follow-up period of 8 years. We have compared the morphologic changes with the cytogenetic and molecular responses at multiple time points during follow-up.

Spectrum of Peripheral Blood and Bone Marrow Changes during TKI Therapy

All CML-CP patients in the study were given 400 mg imatinib mesylate once daily.

Peripheral Blood Changes

TKI therapy in our CML-CP patients was found to be quite effective, with 90% of patients achieving normalization of

Table 3 Follow-up bone marrow findings in baseline chronic-phase patients

Parameter		Months of TKI exposure											
		3	6	12	18	24	30	36	42	48	60	72	84
Bone marrow aspirate, *n*		10	120	128	100	92	49	67	42	38	52	17	14
Particulate aspirate		9 (90%)	81 (66%)	79 (61%)	81 (81%)	74 (80%)	40 (81%)	55 (82%)	37 (88%)	32 (84%)	35 (67%)	11 (64%)	12 (85%)
M:E ratio		1.95 (0.6–10)	2.9 (0–100)	2.8 (1–99)	2.5 (1–23)	2.5 (0.9–50)	3 (1–99)	2.3 (1–99)	3 (1–40)	2.8 (0.8–100)	2.8 (1–100)	2 (0–35)	3 (1–15)
Megakaryocyte hyperplasia		4 (40%)	66 (54%)	6 (5%)	8 (8%)	6 (6%)	1 (2%)	3 (4%)	3 (7%)	4 (10%)	5 (10%)	3 (18%)	1 (7%)
Dwarf megs	<10%	8 (80%)	108 (90%)	115 (90%)	78 (78%)	77 (84%)	43 (88%)	58 (87%)	31 (74%)	32 (84%)	45 (87%)	13 (76%)	13 (93%)
	≥10%	2 (20%)	12 (10%)	13 (10%)	22 (22%)	15 (16%)	6 (12%)	9 (13%)	11 (26%)	6 (16%)	7 (13%)	4 (23%)	1 (7%)
Trephine biopsy, *n*		7	56	63	18	33	20	27	13	14	20	0	5
Cellularity	Hypocellular	3 (43%)	22 (33%)	17 (27%)	3 (17%)	15 (46%)	6 (30%)	7 (26%)	5 (38%)	3 (21%)	5 (25%)	–	1 (20%)
	Hypercellular	2 (29%)	18 (32%)	22 (35%)	5 (28%)	5 (15%)	6 (30%)	10 (37%)	6 (46%)	5 (36%)	4 (20%)	–	–
	Normocellular	2 (29%)	16 (29%)	24 (38%)	10 (56%)	13 (39%)	8 (40%)	10 (37%)	3 (23%)	6 (43%)	11 (55%)	–	4 (80%)
Retic score	<2	NA	5 of 6 (83%)	3 of 5 (60%)	3 of 5 (60%)	5 of 9 (56%)	1 of 4 (25%)	5 of 9 (56%)	3 of 5 (60%)	2 of 4 (50%)	2 of 3 (60%)	–	–
	≥2	NA	1 of 6 (17%)	2 of 5 (40%)	2 of 5 (40%)	4 of 9 (44%)	3 of 4 (75%)	4 of 9 (44%)	2 of 5 (40%)	2 of 4 (50%)	1 of 3 (40%)	–	–
Lymphoid nodule		1 (14%)	4 (4%)	5 (8%)	6 (33%)	2 (6%)	2 (10%)	2 (7%)	0	4 (29%)	1 (5%)	–	0

Abbreviations: M:E, myeloid to erythroid ratio; TKI, tyrosine kinase inhibitor.

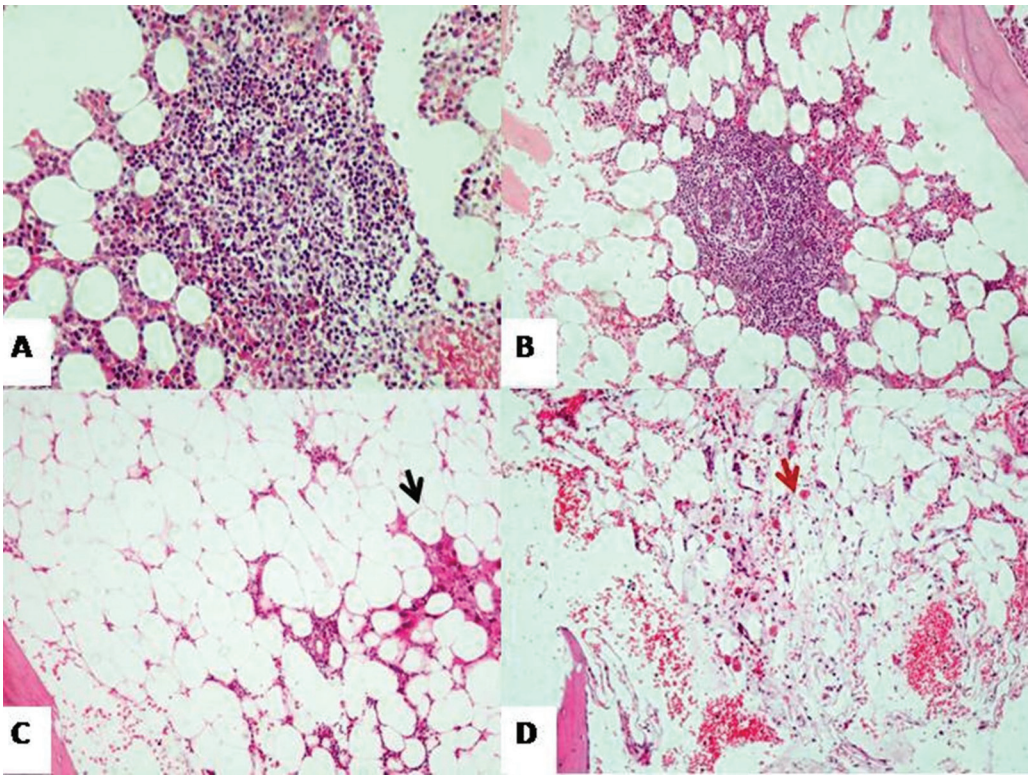

Fig. 2 Hematoxylin and Eosin–stained sections of four baseline CML-CP patients showing morphologic changes observed during TKI therapy. (**A**) CML 69: a 48-year-old male at 48th month of TKI showing interstitial and circumscribed lymphoid nodule (40 × 10 magnification). (**B**) CML 462: a 35-year-old female at 18th month of TKI showing interstitial lymphoid nodule with germinal center formation in a marrow with hypocellularity (20 × 10 magnification). **(C)** CML 120: a 28-year-old male at 6th month of TKI showing hypocellular marrow spaces. Note the focal clustering of megakaryocytes (black arrow; 20 × 10 magnification). (**D**) CML 242: a 44-year-old male at 24th month of TKI showing hypocellular marrow spaces and stromal degeneration (red arrow; 20 × 10 magnification). CML-CP, chronic myeloid leukemia in chronic phase; TKI, tyrosine kinase inhibitor.

Table 4 Disease status among baseline chronic-phase CML patients during follow-up

	Months of TKI therapy											
	3	6	12	18	24	30	36	42	48	60	72	84
Number of patients	10	120	128	100	92	49	67	42	38	52	17	14
Complete hematological response	9 (90%)	98 (82%)	101 (79%)	87 (87%)	73 (79%)	38 (78%)	51 (76%)	29 (69%)	31 (81%)	37 (71%)	15 (88%)	12 (86%)
Not in complete hematological response.	–	15 (12%)	21 (16%)	12 (12%)	15 (16%)	6 (12%)	10 (15%)	8 (19%)	5 (13%)	14 (30%)	2 (12%)	2 (14%)
Accelerated phase	–	3 (3%)	3 (2%)	–	–	–	3 (4%)	3 (7%)	–	–	–	–
Blast crisis	1 (10%)	4 (3%)	3 (2%)	1 (1%)	4 (4%)	5 (10%)	3 (4%)	2 (5%)	2 (5%)	1 (2%)	–	–

Abbreviations: CML, chronic myeloid leukemia; TKI, tyrosine kinase inhibitor.

TLC, platelets, and peripheral blood differential counts by the third month of TKI therapy. This hematologic remission was maintained in nearly 80% of patients during the subsequent follow-up time points till 8 years.

Cytopenias during Follow-Up

With an overall incidence of 25%, anemia was the most common cytopenia seen at all follow-up time points (range: 14% patients at 42nd month and 40% patients at 6th and 52nd months). This is similar to the inference obtained by Paul et al from India.[12]

Bone Marrow Changes during Follow-Up

In tandem with the normalization of TLC at 3rd month of TKI, there was normalization of marrow cellularity and granulocytic series of cells by 3rd month (M:E ratio range at 3rd month: 0.6:1–10:1). This rapid normalization of marrow cellularity (by 13th week of imatinib) was also observed in European literature.[7]

Marrow hypocellularity was seen in nearly 20 to 40% of our patients during overall follow up, the incidence ranging from 12% at 42nd months to 43% at 3rd month. Joshi et al have recorded 54% incidence of marrow hypocellularity (37

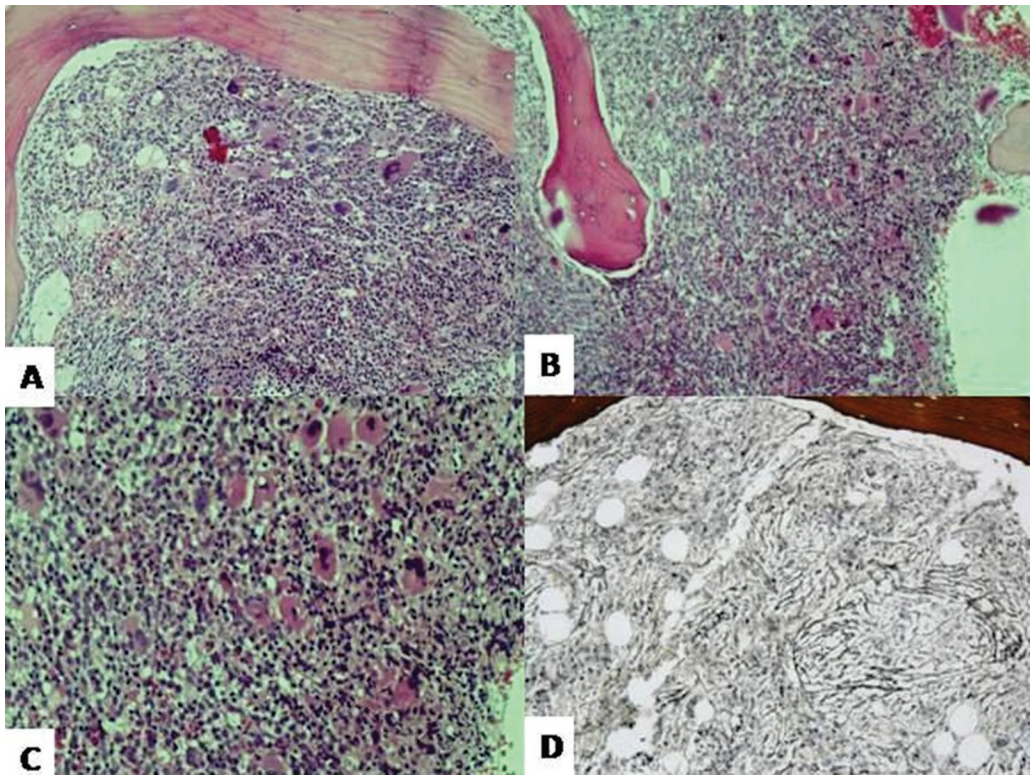

Fig. 3 CML169: a 49-year-old male with loss of CHR and cytogenetic failure at 24th month of Imatinib. (**A**–**C**) H&E-stained bone marrow biopsy sections showing hypercellular marrow spaces with megakaryocytic hyperplasia and clustering. (**C**) Loose clustering of megakaryocytes. (**D**) Reticulin staining showing a modified Bauermeister score of 3, indicating myelofibrosis. (A, B: 20 × 10 magnification; C, D: 40 × 10 magnification). CHR, complete hematological response.

of 68 cases) at 6th month of imatinib therapy[9]; however, the incidence of 6th month marrow hypocellularity in our study is 33%. Srinivas et al have observed marrow hypocellularity in approximately 6% patients on imatinib, though the time point has not been mentioned.[8]

Though the median platelet counts were within normal range as early as 3rd month of TKI therapy, the corresponding normalization of bone marrow megakaryocyte hyperplasia was seen only by 12th month (at 3rd, 6th, and 12th months of TKI therapy, megakaryocytic hyperplasia was seen in 40, 54, and 5% patients, respectively). However, even later normalization of megakaryocyte hyperplasia has been mentioned by a European study, who observed normalization of megakaryocytic hyperplasia by 49th month of TKI therapy.[7]

Therefore, the bone marrow changes observed on TKI therapy include normalization of granulocytic and megakaryocytic hyperplasia by 3rd and 12th months, respectively. Longer duration of TKI therapy caused hypocellularity in 20 to 40% of patients.

Comparison of Peripheral Blood and Bone Marrow Findings with Respect to Hematologic Remission Status of CML-CP Patients

As the hematologic remission of CML patients on follow-up was assessed by ELN 2013 recommendations, there were significant differences in the platelet count, TLC, and blast percentage between the CHR and NCHR groups of patients ($p < 0.001$) as expected.

Among all the peripheral blood and bone marrow parameters analyzed, marrow hypercellularity assessed from trephine biopsy was the only parameter which was consistently significant between the CHR and NCHR responses (p-values ranging from <0.000 to 0.001) till 48 months. Although both ELN and National Comprehensive Cancer Network guidelines require only the peripheral blood sample to segregate CHR and NCHR TKI responses and a bone marrow aspirate to evaluate cytogenetic remission, there are no clear cut guidelines regarding the role of trephine biopsy evaluation during follow-up bone marrows in CML patients on TKI. The significant difference between the CHR and NCHR responses with respect to hypercellular marrow spaces in our cohort indicates the role for trephine biopsy in TKI response assessment.

Even though 80% patients were in CHR at any point of follow-up, dwarf megakaryocytes ($\geq$10%) could be seen in up to 20% cases, at all follow-up time points. This inference is also mirrored by Lugli et al, where in they had also observed the persistence of dwarf megakaryocytes in patients with optimal response.[7]

Therefore, in comparison of CHR and NCHR patients, significant differences were seen in the platelet count, TLC, blast percentages (as expected), and marrow hypercellularity. Also,

persistence of dwarf megakaryocytes was observed in 20% patients.

Comparison of Peripheral Blood and Bone Marrow findings with Respect to Cytogenetic Response Status of CML-CP Patients

On evaluating the cytogenetic response as per ELN 2013 guidelines, none of the peripheral blood and bone marrow parameters were significantly different among the patients with optimal and suboptimal (warning and failure) cytogenetic responses. In fact, the CHR and NCHR responses were also not significant in predicting the cytogenetic remission status.

Conclusion

Normalization of platelet count, TLC, marrow cellularity, and granulocytic hyperplasia occurs by 3rd month of TKI therapy. Even though platelet counts normalize by 3rd month, megakaryocytic hyperplasia in the marrow normalizes by 12th month of TKI therapy only. Cytopenias are invariably seen in all follow-up time points, with thrombocytopenia, being most common therapy induced cytopenia. Marrow hypocellularity was seen in nearly 20% patients during TKI therapy.

At all-time points, marrow hypercellularity is higher in proportion in NCHR patients than those in CHR. None of the peripheral blood and bone marrow parameters analyzed in our study were consistently different between optimal and non-optimal cytogenetic (warning and failure) responders.

Limitation of the Study

There are limitations of this study, including: (1) due to retrospective nature of study, data completeness at all follow-up time points is not there; (2) as there is no fixed bone marrow evaluation schedule for CML patients on TKI therapy, the number of patients is variable at different time points; (3) bone marrow fibrosis and reticulin grading could not be evaluated in all patients; (4) absence of correlation with *BCR::ABL1* levels.

Patient's Consent

The authors certify that they have obtained all appropriate patient consent.

Funding

None.

Conflict of Interest

None declared.

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