Original Article

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Various Treatment Responses in Imatinib Treated CML Patients with Emphasis on Marrow Profile - A Descriptive Study in a Tertiary Care Centre in South India

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ABSTRACT

Background: CML is a clonal stem cell neoplasm characterised by reciprocal translocation between chromosomes 9 and 22. Imatinib is used in its treatment which induces hematological, cytogenetic and molecular response. Yet, marrow response is less well defined than hematological and cytogenetic response (HR and CR). Hence our aims were

1. To study the hematologic response in CML patients after 3 months of Imatinib

2. To study the cytogenetic response in CML patients after 6 months of Imatinib.

3. To study and categorize morphological changes in marrow at the end of three months of treatment.

Materials and Methods: Forty six newly diagnosed patients of CML (BCR-ABL positive), irrespective of phase, were included. HR and CR was evaluated at the end of three months and six months of Imatinib therapy, respectively. A bone marrow aspirate and biopsy were performed at the end of three months, marrow responses were classified and parameters were analysed.

Results: Of 46 patients, 91.3% of patients attained complete hematological response.30/46 patients had a follow up FISH analysis at 6 months, of whom 70% attained complete cytogenetic response (table/figure 3). Twenty-nine (64.5%) of the post treatment aspirates were diluted and imprints/biopsy yielded information. Twenty-eight (63%) patients showed marrow normalization, eleven (23%) persistence of disease, four (8%) progression of disease to blast crisis (BC) and three (6.5%) hypo cellular marrow.

Conclusion: Post therapy BM in CML, when undertaken should have biopsy rather than an aspirate alone, as aspirates are often diluted and biopsy may yield useful information like impending blast crisis.

Keywords: *Hematological and Cytogenetic Response, Marrow Response*

Introduction

CML is a clonal stem cell neoplasm characterised by reciprocal translocation between chromosomes 9 and 22.[1] Imatinib is a novel drug used in its treatment which induces hematological, cytogenetic and molecular response.[2] Yet, marrow response in Imatinib treated CML patients is less well defined than haematological and cytogenetic response (HR and CR) with a paucity of studies from South India.[3,4]

Hence our aims were 1) To study the hematologic response in CML patients after 3months of Imatinib. 2) To study the cytogenetic response in CML patients after 6months of Imatinib. And 3) To study and categorize morphological changes in marrow at the end of three months of treatment

Materials and Methods

This was a descriptive study conducted for a period of two and half years in a tertiary care centre in South India. Forty six newly diagnosed patients of CML (confirmed by BCR-ABL positivity), irrespective of phase, were included in the study. Patients previously treated with other drugs (Busulfan/Interferon/Hydroxyurea) and those with other myeloproliferative neoplasms were excluded. The study was approved by Institute Ethics Committee (EC/2011/4/34).

Clinical details were recorded on admission. Complete blood counts(CBC) peripheral smear and Bone marrow (aspirate and biopsy) was done at diagnosis. Patients received Imatinib mesylate as a single dose (300 to 800 mg per day) after the largest meal of the day. Complete blood counts (with peripheral smear) were monitored weekly for the first month, fortnightly thereafter till patient achieved complete HR and then monthly. After 3 months of Imatinib , a repeat marrow was performed and parameters were compared. Dual colour dual fusion FISH was done at diagnosis to assess BCR-ABL positivity with a follow up FISH at six months to assess

the cytogenetic response.[5] FISH analysis was performed on peripheral blood.

Criteria to assess hematological response :

A. CML-Chronic Phase[5,6]

a. **Complete Hematological Response(CHR):** WBC count <10×10^9/L, platelet count <450×10^9/L, no immature cells (ie, blasts, promyelocytes & myelocytes), absence of splenomegaly for atleast 4 weeks

b. **Partial Hematological Response:** Reduction of WBC & platelet counts to less than 50% of that at diagnosis with platelet less than 450,000 and 50% reduction in spleen size

c. Nil Hematological Response: Worser than partial response

B. CML- Accelerated Phase[7]

a. COMPLETE RESPONSE: Blast < 5% in bone marrow, no blasts in peripheral blood, Absolute Neutrophil Count(ANC) of more than 1500/cmm, platelet count of atleast 1,00,000/cmm and no evidence of extramedullary involvement

b. **Marrow Response:** Blast less than 5% in bone marrow, no blasts in peripheral blood, ANC of atleast 1000/cmm, platelet count at least 20,000/cmm (without platelet transfusion and without evidence of bleeding) and no evidence of extramedullary involvement

c. **Return to Chronic Phase:** Less than 15% myeloblasts in peripheral blood and bone marrow, less than 30% myeloblasts plus promyelocytes in the peripheral smear & bone marrow, less than 20% peripheral basophils and no extramedullary involvement other than in liver or spleen(to check)

Cytogenetic Response was Graded as follows[7]

- complete cytogenetic response : absence of Ph positive cells
- partial cytogenetic response : 1%-35% Ph-positive cells
- minor cytogenetic response : 36%-65% Ph-positive cells
- minimal cytogenetic response : 66%-95% Ph-positive cells
- no response : >95% Ph positive cells

All the aspirate smears were stained by Giemsa and Leishman and independently reviewed by two pathologists. Imprint smears and biopsies were used in case of diluted aspirates. Sections were stained with Hematoxylin and Eosin and parameters studied. Patients were classified at the time of diagnosis into CML granulocytic(CML-G) and CML granulocytic megakaryocytic(CML-GM) types.[8] The latter included cases which had increase in megakaryocytes with clustering, atypia and change in topography (paratrabecular location).Special stains for fibrosis(Reticulin and Masson trichrome) was performed. Anti-CD34 antibody was done to assess angiogenesis by calculating mean vessel density (MVD) and to confirm the number of blasts in marrow. Mean vessel density was calculated by taking the average of neomicrovascular channels in three hot spots[9].CD61 was done in cases to confirm megakaryocytic hyperplasia and clustering.

The marrow status at the end of 3 months were classified as follows [10]

- Normalization of marrow: normalization of cellularity and M:E ratio (according to age), no increase in immature precursors/ blast, abnormal megakaryocytes less than 10%.
- Persistence of disease- marrow is hypercellular with persistence of high M:E ratio, abnormal megakaryocytes more than 10% and absence of clustering of blasts
- Progression of disease- clustering and paratrabecular increase in blasts (confirmed by CD34 positivity), abnormal megakaryocytes(>10%)
- Marrow hypoplasia: hypocellular marrow for age and pancytopenic peripheral counts
- The morphological changes were noted and then correlated with the various treatment responses.

Statistical Analysis: All categorical data was represented as percentages and was compared using chi-square and fishers exact test. Distribution of all continuous variables were tested. The normally distributed variables was represented as mean with standard deviation and median with range was used to express non- gaussian data. To analyse the parameters at diagnosis and at 3 months, paired t test was used for normally distributed variables and non-parametric test for non-gaussian data. Correlation analysis was used to find the association of continuous variables and chi-square test was used to find the association of qualitative variables. Statistical analysis was done using SPSS11.5 software. All statistical analysis was carried out for two tailed significance and p value less than 0.05 was considered significant.

Result

Of 46 patients, 30 were males and 16 females. The median age of patients was 30 years (range17-65 years). Forty three patients were in chronic phase and three in accelerated phase. Biopsy revealed 23 cases of CML-granulocytic megakaryocytic type and 23 cases of CML-

granulocytic type. All patients tested positive for the classic Philadelphia chromosome, except one patient who had a Philadelphia variant. Splenomegaly was the most common presentation of whom 59% had massive spleen. Except three patients, all had varying degrees of splenomegaly. The other clinical symptoms were hepatomegaly (40.4%), pallor(28.3%) and priapism(2.1%).None of the patients had lymphadenopathy at presentation. The mean hb, TLC and platelet count at diagnosis was 9g%, 1,88,066/cmm and 446000/cmm respectively. At diagnosis, all patients had a hypercellular marrow with myeloid hyperplasia comprising predominantly of myelocytes and metamyelocytes. Dwarf megakaryocytes were seen with many sea blue histiocytes.

Various Treatment Responses in CML Patients: After three months of Imatinib, there was a significant decrease in TLC and platelet count, mean basophil and blast% with rise in haemoglobin level in the peripheral blood (table 1). Of 46 patients, 91.3% of patients attained complete hematological response (table 2). 30/46 patients had a follow up FISH analysis at 6 months, of whom 70% attained complete cytogenetic response (table 3). Twenty nine (64.5%) of the post treatment marrow aspirates were diluted and imprints/biopsy yielded information. In the marrow, imatinib caused a significant decrease in marrow cellularity, blast and basophil% . Abnormal megakaryocytes decreased in number, yet not to a significant level(table 4). Fibrosis and MVD decreased post therapy, but p value was not significant.

The marrow response in 46 patients is shown (figure 1&2). 28/46 patients attained marrow normalization. Their marrow revealed decrease in cellularity with normalization of M:E ratio, decrease in megakaryocyte number and normalization of megakaryocyte morphology (persisting dwarf megakaryocytes less than 10%). Fibrosis was less than or equal to 1 in all these cases.11/46 patients had persistence of disease with marrow hypercellularity, persistence of immature precursors but no clustering of blasts and abnormal megakaryocytes (persisting dwarf megakaryocytes more than 10%). 4/46 patients had progression of disease with hypercellular marrow spaces showing clustering of blasts. Of the four, one patient had a diluted marrow and trephine biopsy brought into light the clustering of blasts. 3/46 patients had hypocellular marrow for age with patchy residual hematopoietic islands.

Marrow Fibrosis and MVD: Marrow fibrosis was assessed in 36 trephine biopsies (only) due to various technical reasons. At diagnosis, 17 patients had WHO grade 1 fibrosis, 13 had grade 2 and 6 had grade 3. Post therapy, fibrosis decreased in 11(29%) patients by 1 grade and in 3(8%) patients by 2 grades. 11(29%) patients had grade 1 fibrosis and the same persisted post therapy also (figure 3&4). None of the patients had increase in fibrosis post therapy. To summate, 3 patients had minimal fibrosis (grade 0),22 had grade 1 fibrosis,6 had grade 2 and 5 had grade 3 fibrosis. The mean vessel density at diagnosis was 8.2. After three months of Imatinib, MVD decreased to 7.7, but was not clinically significant (figure 4).

Correlation of Marrow Features with Hematologic Response and Cytogentic Response: There was a significant correlation between the decrease in the mean marrow blast%, basophil% and cellularity with CHR. But no significant correlation was obtained between any of the marrow features with the complete cytogenetic response(CCR) (table 5). When we compared the attainment of CHR among the various marrow responses, all patients(except those with blast crisis) achieved CHR. This is a remarkable and yet another important observation as regards to the patients with marrow non-normalisation.

Follow up: The median follow up was 14 months (range 4-24 months). All patients with marrow normalization had an uneventful course. The patients with persistence of disease attained normal/near normal blood counts on follow up. Three of four patients with progression of disease died and the other lost to follow up. The follow up of three patients with hypocellular marrow was unremarkable with mild transient thrombocytopenia.

Discussion

Imatinib is a novel drug directed against a specific target BCR-ABL tyrosine kinase.[2] There are very few studies from Indian subcontinent which correlates haematological, cytogenetic and marrow response after Imatinib treatment.[4,5,10] In this study, the marrow changes three months post Imatinib were correlated with HR and CR.

Table 1: Hematological response in CML patients

HEMATOLOGICAL RESPONSE	NO.OF PATIENTS(N=46)		PERCENTAGE(%)
	chronic phase	accelerated phase	
COMPLETE RESPONSE	41	1	91.3
PARTIAL/ MARROW RESPONSE	1	1	4.3
NO RESPONSE/RETURN TO CHRONIC PHASE	1	1	4.3

Table 2: Cytogenetic response in CML patients.

CYTOGENETIC RESPONSE	NO.OF PATIENTS(N=30)		PERCENTAGE(%)
	Chronic phase	Accelerated phase	
COMPLETE	20	1	70
PARTIAL	6	1	23.3
MINOR	2	0	6.6
MINIMAL	0	0	0
N0	0	0	0

Table 3:Comparison of pretherapy vs post therapy parameters in marrow.

MARROW PARAMETERS	PRE-THERAPY	POST-THERAPY	p VALUE
HYPERCELLULARITY	100%	25%	0.0001
MEAN BLAST%	3.2	0.13	0.000
MEAN BASOPHIL %	6.8	0.49	0.000
ABNORMAL MEGAKARYOCYTES(>10%)	92%	71%	0.347
MVD	8.2	7.7	0.649

Table 4: Correlation of marrow parameters (post therapy) with haematological response and cytogenetic response

BM Parameters (post therapy)		CHR		p value	CCR		p value
		Achieved	Not achieved		Achieved	Not achieved	
Marrow blast	<5% >5%	32 1	3 1	0.000	17 0	6 1	0.342
Marrow basophils	<2% >2%	33 0	1 3	0.000	15 2	6 1	0.904
Cellularity	Normocellular Hyper and hypocellular	33 9	0 4	0.001	16 5	5 4	0.216
Abnormal Megakaryocytes	Less than 10% More than 10%	29 13	4 0	0.139	15 6	6 3	0.782
Fibrosis (WHO grade)	Less than 2 2 and above	4 38	0 4	0.179	2 19	1 8	0.887
Mean vessel density	<10 >10	32 10	3 1	0.779	19 2	6 3	0.522

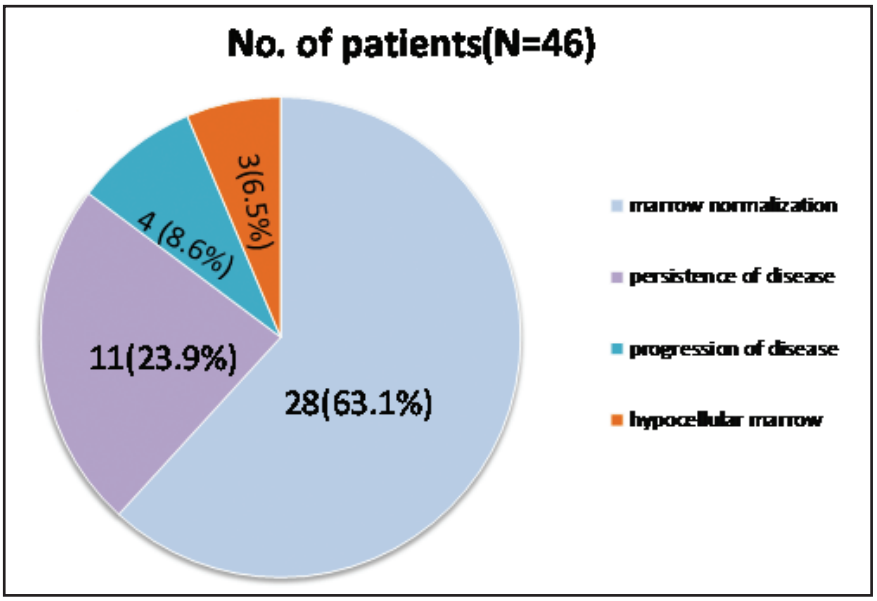

Fig. 1: Marrow response after 3 months of Imatinib

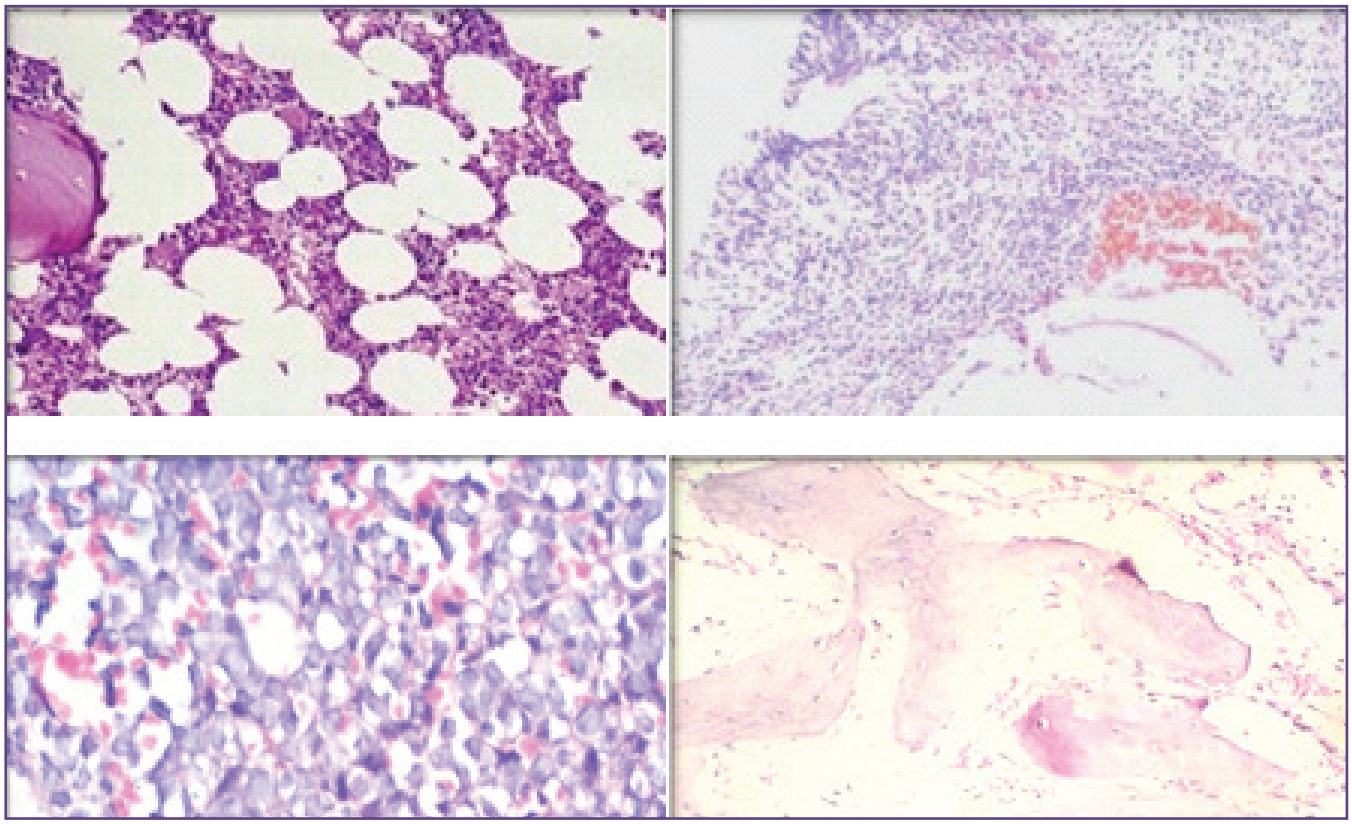

Fig. 2: Trephine biopsy showing (a) marrow normalization (H&E, x100) (b) persistence of disease(H&E, x100) (c) progression of disease (H&E, x400) and (d) hypocellular marrow with neo-osteogenesis(H&E, x100).

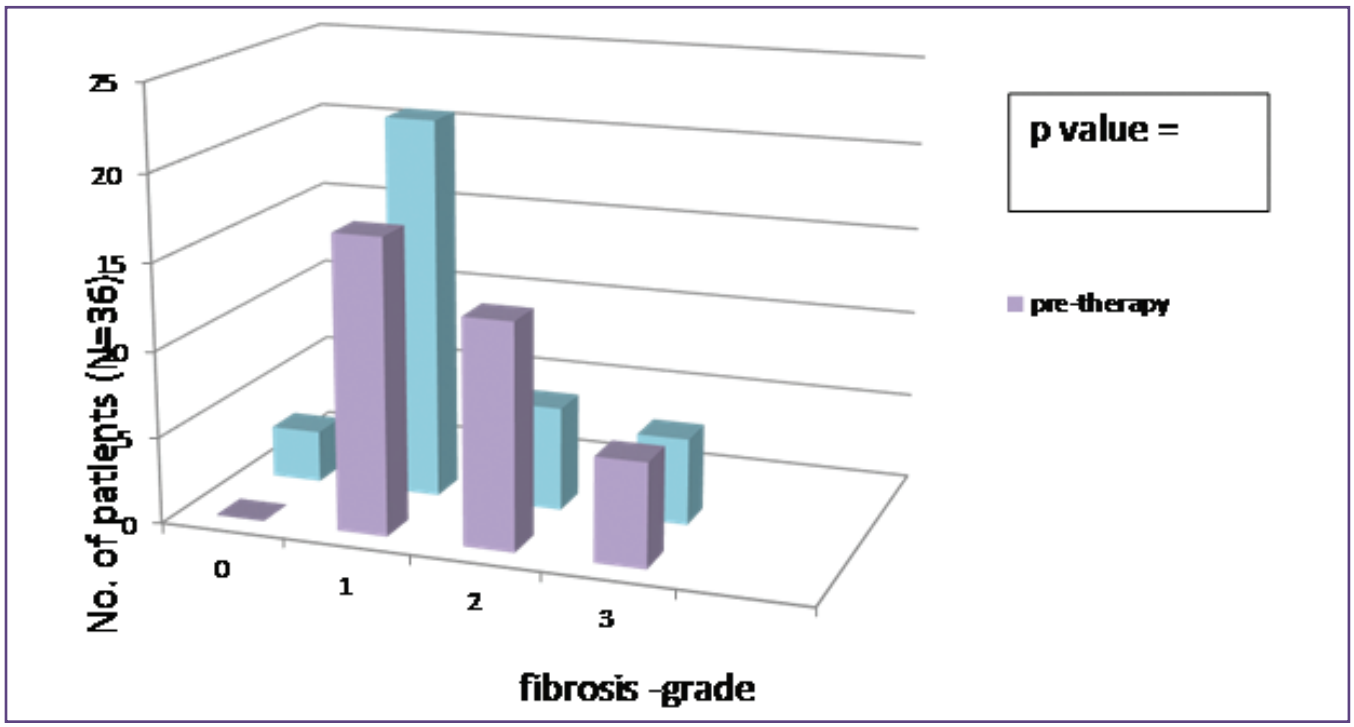

Fig. 3: Comparison of pretherapy and post therapy marrow fibrosis

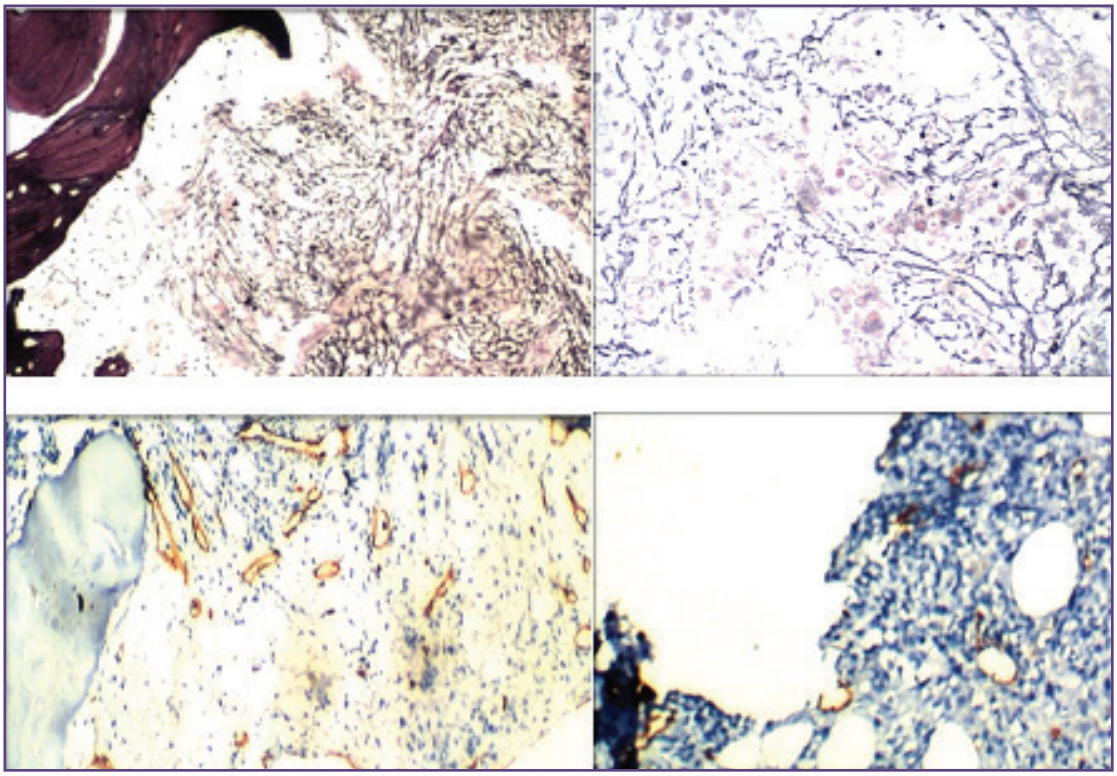

Fig. 4: Paired trephine biopsies of two sets of patients showing (a) WHO grade 3 fibrosis at diagnosis (Reticulin stain, x100) which decreased to (b) WHO grade 1 fibrosis post therapy (Reticulin stain, x100) (c) raised MVD at diagnosis (CD34 IHC, x400) and (d) normalization of MVD post therapy(CD34 IHC,x400)

Imatinib induces durable hematologic and cytogenetic responses in patients of CML, though the former is achieved earlier. Ninety percent of our patients achieved CHR while only 70% of them attained complete cytogenetic response. Other studies support the same.[6,11,12]Studies also quote that CCR occurred in the setting of nearly universal haematological response.[11] In our study except for two, all patients who achieved CHR also achieved complete CCgR.

Dilution of post therapy aspirates is known.[13] In this study,29 patients (64.5%)had a diluted marrow aspirate, including a patient who showed impending BC in marrow trephine. Hence dilution in post treatment marrows should be dealt with caution. Of 18 patients who did not achieve marrow normalisation, 14 achieved CHR. Hence non-normalization of marrow in the form of persisting hypercellularity, presence of immature precursors (not blasts) and abnormal megakaryocytes need not be worrisome as long as CHR is achieved, as it is well known that marrow response lags behind haematological response. [7]Most of these marrows normalize in a couple of months as evidenced indirectly by the normalising peripheral counts. All patients had an unremarkable course except for those in blast progression. Varying degrees of cytopenias do occur in patients of CML on Imatinib, though most of them are transient and mild.[10]

Though most of the patients have the classical Philadelphia chromosome, Philadelphia variants do occur in patients of CML. Imatinib is known to provide durable hematological responses in such patients too[14]. In our study, only one had a Philadephia variant. This patient achieved complete hematological and cytogenetic response and the follow up was uneventful.

Imatinib reduces bone marrow fibrosis, in contrast to other therapeutic drugs used earlier like interferon alpha, which were fibrogenic.[15] In our study, fibrosis decreased post therapy, though not significantly. This could well be attributed to the persistence of abnormal megakaryocytes, the source of marrow PDGF and hence fibrosis.[16] It could be argued that the elevated vessel counts recorded in CML may be attributable to reactivation of preexisting functionally dormant sinusoid endothelium. The distinctly abnormal architecture of the microvessels with increased tortuosity speaks in favor of a truly neoangiogenic phenomenon elicited in response to increased VEGF production from elevated leukocyte numbers in the peripheral blood as well

as in the bone marrow.[9] Studies have shown that VEGF expression is highest in megakaryocytes[17] and serum VEGF levels are highest in CML than other leukemias.[18]

Our study has few limitations. Serial aspirates and biopsies were not done owing to the invasiveness of the marrow procedures. Cytogenetic response was assessed in peripheral blood(not in marrow aspirates), which would have given better results.

Conclusion

We conclude saying that follow up marrows in CML patients on treatment is not mandatory. Post–therapy marrow aspirates are known to have a poor yield because of dilution by peripheral blood. For centres practicing a follow-up marrow as a protocol, it is emphasized to do a bone marrow biopsy rather than aspirate alone, to overcome the disadvantage of dilution.

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