Original Research Article

LONG-TERM EVALUATION OF IMATINIB´S EFFECT ON BONE MARROW FIBROSIS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

Hosny Badrawy Hamed;[1] Asmaa Mohamed Zahran,[2] Adel Gomaa,[3] Noha Gaber Sayed,[4] Ahmed Makboul[5]

1. Professor of Clinical Pathology, South Egypt Cancer Institute, Assiut University.

2. Assistant Professor of Clinical Pathology, South Egypt Cancer Institute, Assiut University.

3. Assistant Professor of Clinical Oncology, South Egypt Cancer Institute, Assiut University.

4. Lecturer of Clinical Pathology, South Egypt Cancer Institute, Assiut University.

5. Assistant Lecturer of Clinical Pathology, South Egypt Cancer Institute, Assiut University. E-mail address: a.makboul1987@gmail.com

Abstract:

Background: Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm (MPN) that originates in an abnormal pluripotent stem cell **[1].** At diagnosis, all cases of CML have the characteristic t(9;22) translocation that results in the Ph chromosome **[2].** Marked bone marrow (BM) fibrosis at the time of diagnosis and worsening BM fibrosis while the patient is receiving therapy are markers of CML disease progression and poor prognosis **[3].** A significant advance in the treatment of CML was achieved with the introduction of imatinib **[4].** Imatinib has an independent anti-fibrotic effect on BM of CML patients **[5].**

Aim of the study: We assessed BM fibrosis in CML patients treated with imatinib.

Patients and methods: This retrospective study was done on 46 patients with CML from 2012 to 2014 treated with imatinib. Assessment of reticulin fibrosis in CML patients treated with imatinib was done using reticulum staining kit.

Results: Thirty-three patients (71.7%) of the 46 patients in our study showed progression of BM fibrosis after one year of treatment with imatinib.

Conclusion: Our findings indicate that imatinib may be associated with progression of BM fibrosis in CML patients.

Keywords:

Chronic myeloid leukemia; imatinib; bone marrow fibrosis.

Introduction:

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm (MPN) that originates in an abnormal pluripotent bone marrow (BM) stem cell **[1].** The presence of a balanced translocation between chromosome 9 and chromosome 22, t(9;22) (q34;q11) is the basis of the diagnosis and a hallmark for treatment of CML **[2].** The natural history includes: an initial indolent chronic phase (CP) that is followed by an accelerated phase (AP), a blast phase (BP) or both **[1].**

Improvements in the understanding of the molecular mechanism underlying CML has led to the evolution of targeted therapies. In the early 1990s, Lyndon and Matter worked on the development of specific tyrosine kinase inhibitors (TKI). From this drug discovery program, imatinib was generated **[6].**

Imatinib, a first generation TKI, is the cornerstone in the treatment of CML **[7]**. Imatinib functions as a competitive inhibitor of the *BCR-ABL* tyrosine kinase leading to inhibition of proliferation, restoration of cell cycle control, induction of apoptosis and reversal of genetic instability in *BCR-ABL* dependent cells in vitro **[8]**. Imatinib was initially approved by the Food and Drug Administration (FDA) for the treatment of advanced CML (AP or BP or in CP after interferon-α failure) in the year 2001. It was then approved as a first line treatment for CP-CML in the year 2002 **[9].**

Bone marrow (BM) fibrosis is the continuous replacement of blood-forming cells in the BM by excessive scar tissue, leading to failure to produce blood cells and ultimately to death **[10]**. BM fibrosis at diagnosis of CML, or its later development during the course of the disease, is a poor prognostic factor. BM reticulin fibrosis occurs in 40% of patients with CML at diagnosis **[5].**

The fibrogenic effects of CML may result from contact-mediated effects of *BCR-ABL* hematopoietic cells on BM stromal cells as well as production of platelet derived growth factor (PDGF) by CML cells **[11].**

Imatinib has an independent anti-fibrotic effect on BM of CML patients. It acts by blocking the binding site of *PDGF*, present in fibroblasts that are involved in the release of various elements, such as reticulin. This significant resolution of BM fibrosis with imatinib therapy may improve prognosis in CML through mechanisms either independent of or related to suppression of *BCR-ABL* **[5].** An increase in angiogenesis has been observed in patients with hematological diseases including CML **[12].** One study investigated the role of imatinib mesylate on BM angiogenesis. This study reported that – in vitro- the expression of vascular endothelial growth factor (VEGF) reduced after treatment with imatinib. Also, it suggested that the decrease in micro vascularization presents an association with the reversal of BM fibrosis **[13].**

Although most studies showed that imatinib reduces BM fibrosis in CML patients, some studies showed that imatinib does not absolutely guarantee against evolution of BM fibrosis **[14].** A study reported that expansion of JAK-2 mutated clone (V617F) is a possible mechanism of emerging BM fibrosis during imatinib therapy **[15].**

Because different studies showed that imatinib has different effect on BM fibrosis, the present study aimed to evaluate BM fibrosis in CML patients treated with imatinib.

Patients and methods:

Patients' characteristics:

This study was done in Clinical Pathology Department, South Egypt Cancer Institute, Assiut University, from 2012 to 2014. The study included 46 patients diagnosed as CML depending on clinical, morphological and cytogenetic/molecular data according to WHO criteria. All patients were under treatment by imatinib (Gleevec ®) (400 mg/day). Age of the patients ranged from 20:60 year with mean age of (39.65 ± 12.7). According to sex, 22 cases were males and 24 cases were females. At diagnosis, all patients were in CP. After one year of treatment, patients were classified into CP, AP or BP based on the WHO criteria **[16]**.

The study was approved by the Ethical Committee of Faculty of Medicine, Assiut University (IRB no: IRB00008718). Informed consents were taken from the patients before enrollment in this study.

Inclusion Criteria:

Patients diagnosed as CML (Philadelphia positive) in chronic, accelerated or blast phase with follow-up BM aspirate/biopsy and follow-up cytogenetics and molecular results were included in this study.

Exclusion Criteria: Patients previously treated with any other drugs (Busulfan/ Interferon/Hydroxurea)

Specimens:

Bone marrow biopsy specimens were obtained. All specimens fixed in 10% formalin, embedded in paraffin and stained with hematoxylin and eosin for morphological assessment of disease stage and fibrous tissue extension.

Methods:

Collected patient data:

1. Clinical examination data.
2. Complete Blood Count (CBC): using automated hematology analyzers (Abbott Cell Dyn Ruby and Abbott Cell Dyn 1700).
3. Bone Marrow Aspiration (BMA).
4. Bone Marrow Biopsy (BMB).
5. Philadelphia Chromosome analysis by Fluorescent in Situ Hybridization (FISH).
6. Quantitative *BCR –ABL* assay by quantitative real time Polymerase Chain Reaction (qRT-PCR).

Definitions of response **[17]**:

1. *Hematologic response:*

Complete: It is defined as:

- Platelet count < 450 x 10^9/L.
- White blood cell count < 10 x 10^9/L, differential without immature granulocytes and with less than 5% basophils.

- Non-palpable spleen.

Partial: It is defined as above except for

- Presence of immature cells.
- Platelet count > 450 x 10^9/L but < 50% of pretreatment.
- Persistent splenomegaly but < 50% pretreatment size.

Cytogenetic response:

- **Complete:** No Ph^+ metaphases.
- **Major:** 0% to 35% Ph^+ metaphases.
- **Partial:** 1% to 35% Ph^+ metaphases.
- **Minor:** 36% to 95% Ph^+ metaphases.
- **None:** Ph^+ > 95%.

Molecular response:

- **Complete:** *BCR-ABL* mRNA transcript nonquantifiable and nondetectable by qRT-PCR.
- **Major:** more than or equal to 3 log reduction of *BCR-ABL* mRNA transcript.

Evaluation of BM fibrosis:

BM reticulin fibrosis staining of BM biopsy sections. Assessment of reticulin fibrosis in CML patients treated with imatinib was done using reticulum staining kit **(ScyTek Laboratories Inc., Logan, Utah, USA)**. Staining method was done as manufacturing protocol. Histological sections of BMB were examined using a light microscope. The entire section was scanned at 10x magnification. Reticulin fibers were graded as MF-0 to MF-3. The classification used was the

European Consensus on grading of BM fibrosis **[18]**. MF-0 indicates no fibrosis **(figure 01 – panel A)**. MF-1 corresponds to loose network of reticulin fibers with many intersections especially in the perivascular area **(figure 01 – panel B)**. MF-2 corresponds to diffuse and dense increase in reticulin fibers with extensive intersections, and occasionally focal bundles of collagen **(figure 01 – panel C)**. MF-3 corresponds to dense increase in reticulin fibers with extensive intersections and coarse bundles of collagen **(figure 01 – panel D)**.

Statistical Analysis:

Statistical analysis was performed using IBM SPSS version-16 (2007). Data were presented as mean ± standard deviation (SD). Clinical signs were expressed as number of positive cases (%). Statistical differences were estimated by means of the independent t-test, one-way ANOVA test and Pearson Chi-Square test. P value of <0.05 was considered significant.

Results:

Demographic and clinical characteristics (table 1):

At presentation, the median age was 44 years and the range was from 20 years to 60 years. As regarding gender, 22 patients (47.8%) were males and 24 patients (52.2%) were females with male to female ratio was 0.9. As regarding clinical data, 18 patients (39.1%) had moderate splenomegaly at presentation and 28 patients (60.9%) had huge splenomegaly.

After one year of treatment, the median age was 45 years. As regarding clinical data, 18 patients (39.1%) showed reduction in the size of spleen, 10 of them (21.7%) showed no splenomegaly and 8 patients (17.4%) showed mild splenomegaly. Sixteen patients (34.8%) had moderate splenomegaly and 12 patients (26.1%) had huge splenomegaly. After one year of treatment, 38

patients (82.6 %) were in CP, 2 patients (4.3 %) were in AP and 6 patients (13.1 %) transformed to BP.

Evaluation of bone marrow (BM) fibrosis (table 2):

Regarding BM fibrosis at presentation, 14 patients (30.4%) had no BM fibrosis (MF-0) and 32 patients (69.6%) had BM fibrosis. Patients with BM fibrosis at presentation were divided as follows: 24 patients (52.2%) had MF-1 and 8 patients (17.4%) had MF-2. BM fibrosis was reassessed in all patients after one year of treatment with imatinib. 36 patients (78.3%) had BM fibrosis after one year of treatment. They were divided into: 8 patients (17.4%) had MF-1, 14 patients (30.45%) had MF-2 and 14 patients (30.45%) had MF-3.

In our study, we also assessed grades of BM fibrosis at presentation and after one year of treatment where we found that, there is a significant progression of BM fibrosis toward MF-3 in CML patients after one year of treatment with imatinib. Thirty-three, patients (71.7%) showed progression of BM fibrosis after one year of treatment with imatinib. Of those, 23 patients had BM fibrosis at presentation and 10 patients were MF-0 at presentation (with no BM fibrosis). Of the 23 patients who had BM fibrosis at presentation, 15 patients showed progression by one degree and 8 patients showed progression by two degrees. The 10 patients who were MF-0 at presentation progressed as follows; 5 patients showed progression by one degree (to MF-1), 2 patients progressed by two degrees (to MF-2) and 3 patients progressed by three degrees (to MF-3) after one year of treatment.

Relations between BM fibrosis and clinical and hematological characteristics (table 3):

In our study, we found that both WBCs and platelet count increased with the advance of BM fibrosis both before and after treatment. Also, PB blast count was found to increase with the advance of BM fibrosis before treatment, where it tends to be stable even with progression of

BM fibrosis after treatment. As regarding basophils count, we found an increase in basophils count in accordance with the increase of BM fibrosis stage with manifested increase in their count in later stages than in early stage of fibrosis after treatment for both PB & BM basophils. We also assessed BM fibrosis at presentation in relation to age of patients and size of spleen at presentation. We found that age of patients and size of spleen may affect BM fibrosis at presentation as there was statistical significance between BM fibrosis and age of patients **(p= <0.001)** and size of spleen at presentation **(p= 0.009).**

Prognosis of patients and BM fibrosis at presentation (table 4):

Prognosis of patients was assessed using three scoring systems, Sokal score, Hasford score and European Treatment Outcome Study (EUTOS). Regarding Sokal score, 10 patients (21.7%) were low risk, 18 patients (39.15%) were intermediate risk and 18 patients (39.15%) were high risk. Regarding Hasford score, 10 patients (21.75%) were low risk, 26 patients (56.5%) were intermediate risk and 10 patients (21.75%) were high risk. Regarding EUTOS score, 38 patients (82.6%) were low risk and 8 patients (17.4%) were high risk.

Relation between BM fibrosis after one year of treatment and patients' response (table 5):

All the patients were evaluated as regarding their response to treatment (clinical and hematological, cytogenetic and molecular). Of all patients, (18) patients (39.1%) showed complete clinical and hematological response while (28) patients (60.9%) showed partial response. Regarding cytogenetic response, (16) patients (34.8%) showed complete response, (2) patients (4.3%) showed major response, (20) patients (43.5%) showed partial response and (8) patients (17.4%) showed no cytogenetic response. As regarding molecular response, (16) patients

(34.8%) showed complete molecular response while (30) patients (65.2%) showed major molecular response. Assessment of BM fibrosis after one year of treatment in relation to patients' response revealed the following: for complete clinical and hematological response, 5 patients (10.9%) were MF-0 (no fibrosis), 5 patients (10.9%) were MF-1, 4 patients (8.6%) were MF-2 and 4 patients (8.6%) were MF-3. As regarding progressive disease (partial response), 5 patients (10.9%) were MF-0 (no fibrosis), 3 patients (6.5%) were MF-1, 10 patients (21.7%) were MF-2 and 10 patients (21.7%) were MF-3. Regarding cytogenetic response, 5 patients (10.9%) with MF-0 (no fibrosis), 3 patients (6.5%) with MF-1, 4 patients (8.6%) with MF-2 and 4 patients (8.6%) with MF-3 showed complete cytogenetic response. One patient (2.2%) with MF-2 and one patient (2.2%) with MF-3 showed major cytogenetic response. Five patients (10.9%) with MF-0 (no fibrosis), 5 patients (10.9%) with MF-1, 4 patients (8.6%) with MF-2 and 6 patients (13%) with MF-3 showed partial cytogenetic response. Five patients (10.9%) with MF-2 and 3 patients (6.5%) with MF-3 showed no cytogenetic response. These findings are not statistically significant in relation to the BM fibrosis.

Relation between grades of BM fibrosis before and after one year of treatment (table 6):

There was a very high statistical significant difference regarding MF-3 at presentation and after one year of treatment **(*P*< 0.001)**, where no cases were presented as MF-3 at presentation however, 14 cases were assessed as MF-3 after treatment.

Discussion:

In our study, 69.6% had BM fibrosis at presentation. **Buesche et al. (2003)** reported that BM fibrosis at presentation was detected in about 30% of patients before treatment and **Kantarjian et al. (2005)** reported 40% of patients had BM fibrosis at presentation **[5] [19]**. The higher

percentage in our study can be attributed to the lower number of cases we assessed (46 cases) compared to Buesche (400 cases) and Kantarjian (110 patients).

In our study, we also assessed grades of BM fibrosis at presentation and after one year of treatment where we found that, there is a significant progression of BM fibrosis toward MF-3 in CML patients under treatment with imatinib. These findings are in agreement with **Buesche et al. (2007)** who stated that imatinib does not absolutely guarantee against evolution of BM fibrosis. They reported that, during imatinib therapy, small foci of fibers emerged in 14 patients; in 6 without any pretreatment and in 8 after pretreatment with interferon-α. Full blown fibrosis emerged in 8 patients. In 2 out of the 8 patients, BM fibrosis reversed after increase of imatinib dose and in the remaining patients, fibrosis did not reverse **[14]**. **Buesche et al. (2007)** had stated that; a loss of the inhibitory effect on PDGFR protein kinase activity might explain the emergence of foci with fiber increase during a cytogenetic and molecular response **[14]**. Also, **Bueso-Ramos et al. (2004)** reported that, the development of BM fibrosis (increased reticulin by at least 2 degrees) was noted as a late event during imatinib therapy (median of 5 months) in 13% of patients who began treatment with no significant BM fibrosis **[3]**. **Vigna et al. (2017)** reported a case that was diagnosed as CML with progression of BM fibrosis when treated with imatinib and the fibrosis gradually disappeared under dasatinib therapy **[20]**. Another explanation of the possible cause of the genesis of fibrosis in CML patients is the demonstration that; megakaryocyte homogenates are able to stimulate the proliferation of BM fibroblasts and the production of collagen has provided the first tangible evidence in support of the role of megakaryocytes and of an megakaryocyte-derived growth factor identified as PDGF, in the genesis of BM fibrosis **[21]**.

In the present study out of the 32 patients who had BM fibrosis at presentation, 8 patients (25%) showed regression of BM fibrosis, 5 patients showed regression by one degree and 3 patients showed regression by two degrees.

These results are in accordance but in lower percentage with **Kantarjian et al. (2005)** study which examined 31 CML patients with severe BM fibrosis at presentation and following imatinib therapy for 3 to 24 months, 61% had significant reduction (at least 2 degrees) of BM fibrosis **[5]**. **Bueso-Ramos et al (2004)** reported resolution of at least 2 degrees in 61% and by at least 1 degree in 85% **[3]**. **Beham-Schmid et al. (2002)** noted the resolution of BM fibrosis with imatinib in 78% of patients with CML they studied **[22]**. **Hasserjian et al. (2002)** studied 21 patients with CML who were MF-2 and MF-3 and observed that, following a median of 3 months of imatinib therapy, they found a significant resolution of BM fibrosis in 15 patients (71%) **[11]**. Also, **McNamara et al. (2003)** observed a reduction in BM fibrosis in 73% of CML chronic phase patients **[23]**. **Narang N et al. (2017)** evaluated BM fibrosis in 60 patients prior to imatinib therapy and were able to evaluate fibrosis grades in 48 patients at 6 months of follow-up and 30 patients at 12 months of follow-up. They observed significant improvements in fibrosis grades at 6 months which continued at 12 months of follow-up **[7]**. The lower percentage in our study may be attributed to the longer follow up period after treatment in our study in comparison with shorter periods in the other studies.

Our study revealed that, the incidence and grade of BM fibrosis increases with old age **($p < 0.001$)**. These findings are in agreement with **Kantarjian et al, (2005)** who revealed a statistical significant relation between BM fibrosis and age of the patients at presentation **[5]**.

Regarding the relation between BM fibrosis and hematological findings, our results revealed that, WBCs and platelet count increase with the advance of BM fibrosis both before and after

treatment. On the other hand, BM blasts show an evident increase in their count mainly after treatment with the advance of the stage of fibrosis. However, these results are statistically insignificant, they found to be matched concerning BM blast and contradirectory concerning WBCs and platelet count to that of **Thiele and Kvasnicka** who stated that, the higher grades of BM fibrosis were associated with increase in blast, low platelets and leucocyte count **[13]**.
For the basophils count, we found an increase in its count in accordance with the increase of fibrosis stage with manifested increase in their count in later stages than in early stage of fibrosis after treatment for both PB & BM basophils. However, these results were statistically insignificant, they found to be in agreement with **Kantarjian et al. (2005)** who reported a statistical significant relation between BM fibrosis and increase in BM basophils count **[5]**.
The assumption of that BM fibrosis at presentation may be associated with poor prognosis could be supported by the presence of statistical significance between BM fibrosis and Sokal score **(P= 0.047).** This was also reported by **Kantarjian et al (2005)** indicating that marked BM fibrosis is associated with high risk Sokal risk groups **[5]**.

Regarding response to treatment, we found that decreased response to treatment was found to be evident in patients with advanced stages of fibrosis ≥ 2. These findings for the clinical, cytogenetic and molecular response assessment. However, no statistical significant relation between BM fibrosis after one year of treatment and response, but our findings were in agreement with **Bueso-Ramos et al. (2004)** as they reported no evident correlation between the degree of BM fibrosis and cytogenetic response **[3]. Hasserjian et al. (2002)** also found that reduction of BM fibrosis with imatinib was independent of the degree of cytogenetic response **[11].** On the contrary, **Jesus et al. (2011)** reported a statistical significant correlation between reduction of BM fibrosis after treatment and cytogenetic response as they found that among the

patients who had their BM fibrosis graded as MF-0, 90% of them showed cytogenetic response **[24]**.

These findings could be explained by the theory adopted by **Klion et al., (2004)** which stated that, in CML, there is a change in the distribution of elements present in the extracellular matrix of BM, and it has been suggested that the leukemic cells are protected or "hidden" by these elements. The increased fibrosis could hinder the performance of the drug, not allowing the apoptosis of Philadelphia chromosome positive cells **[25]**. **Klion et al.,** study suggested that in samples where there was a reduction of fibrosis, the drug worked more easily on the leukemic cells. As the imatinib acts on the stroma of BM, there is the possibility that the reduction of fibrosis provides better conditions for the performance of the drug **[25]**.

Conclusion:

In conclusion, the development of various degrees of BM fibrosis is common in patients with CML. Worse BM fibrosis at the time of diagnosis and worsening BM fibrosis while the patient is receiving therapy are markers of CML disease progression and poor prognosis.
Our findings indicate that imatinib may be associated with progression of BM fibrosis in CML patients on the long run of follow up.

Recommendations:

From our study, we recommend that a larger study shall be done on a larger number of CML patients treated with imatinib and these patients shall be followed up over a longer duration to assess BM fibrosis.

In addition, further studies shall be implemented to compare imatinib with second and third generation tyrosine kinase inhibitors as regarding their effect on BM fibrosis.

Conflict of interest:

The authors declare no conflict of interest.

References:

1. Vardiman, J.W., J.V. Melo, M. Baccarani, J.R. Radich, and H.M. Kvasnicka, *Chronic Myeloid Leukemia, BCR-ABL1 Positive*, in *WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues*. 2017, International Agency for Research on Cancer (lARC): 69372 Lyon Cedex 08, France.
2. Hurtado, R.M., P.V. Vargas, and J.F. Cortes, *Chronic Myeloid Leukemia Current Concepts in Physiopathology and Treatment.* Cancerología, 2007. **2**: p. 137-147.
3. Bueso-Ramos, C.E., J. Cortes, M. Talpaz, S. O'Brien, F. Giles, M.B. Rios, L.J. Medeiros, and H. Kantarjian, *Imatinib mesylate therapy reduces bone marrow fibrosis in patients with chronic myelogenous leukemia.* Cancer, 2004. **101**(2): p. 332-6.
4. Peggs, K. and S. Mackinnon, *Imatinib mesylate--the new gold standard for treatment of chronic myeloid leukemia.* N Engl J Med, 2003. **348**(11): p. 1048-50.
5. Kantarjian, H.M., C.E. Bueso-Ramos, M. Talpaz, S. O'Brien, F. Giles, S. Faderl, W. Wierda, M.B. Rios, J. Shan, and J. Cortes, *Significance of myelofibrosis in early chronic-phase, chronic myelogenous leukemia on imatinib mesylate therapy.* Cancer, 2005. **104**(4): p. 777-80.

6. Henkes, M., H. van der Kuip, and W.E. Aulitzky, *Therapeutic options for chronic myeloid leukemia: focus on imatinib (Glivec, Gleevectrade mark).* Ther Clin Risk Manag, 2008. **4**(1): p. 163-87.

7. Narang, N.C., U. Rusia, M. Sikka, and M. Kotru, *Morphological Changes in Bone Marrow Post Imatinib Therapy in Chronic Phase CML: A Follow up Study on Sequential Bone Marrow Aspirates and Biopsies.* J Clin Diagn Res, 2017. **11**(4): p. EC25-EC29.

8. van der Kuip, H., A. Moehring, L. Wohlbold, C. Miething, J. Duyster, and W.E. Aulitzky, *Imatinib mesylate (STI571) prevents the mutator phenotype of Bcr-Abl in hematopoietic cell lines.* Leuk Res, 2004. **28**(4): p. 405-8.

9. Cohen, M.H., G. Williams, J.R. Johnson, J. Duan, J. Gobburu, A. Rahman, K. Benson, J. Leighton, S.K. Kim, R. Wood, M. Rothmann, G. Chen, K.M. U, A.M. Staten, and R. Pazdur, *Approval summary for imatinib mesylate capsules in the treatment of chronic myelogenous leukemia.* Clin Cancer Res, 2002. **8**(5): p. 935-42.

10. Gleitz, H.F., R. Kramann, and R.K. Schneider, *Understanding deregulated cellular and molecular dynamics in the haematopoietic stem cell niche to develop novel therapeutics for bone marrow fibrosis.* J Pathol, 2018. **245**(2): p. 138-146.

11. Hasserjian, R.P., F. Boecklin, S. Parker, A. Chase, S. Dhar, M. Zaiac, E. Olavarria, I. Lampert, K. Henry, J.F. Apperley, and J.M. Goldman, *ST1571 (imatinib mesylate) reduces bone marrow cellularity and normalizes morphologic features irrespective of cytogenetic response.* Am J Clin Pathol, 2002. **117**(3): p. 360-7.

12. Lundberg, L.G., R. Lerner, P. Sundelin, R. Rogers, J. Folkman, and J. Palmblad, *Bone marrow in polycythemia vera, chronic myelocytic leukemia, and myelofibrosis has an increased vascularity.* Am J Pathol, 2000. **157**(1): p. 15-9.

13. Kvasnicka, H.M., J. Thiele, P. Staib, A. Schmitt-Graeff, M. Griesshammer, J. Klose, K. Engels, and S. Kriener, *Reversal of bone marrow angiogenesis in chronic myeloid leukemia following imatinib mesylate (STI571) therapy.* Blood, 2004. **103**(9): p. 3549-51.

14. Buesche, G., A. Ganser, B. Schlegelberger, N. von Neuhoff, D. Gadzicki, H. Hecker, O. Bock, B. Frye, and H. Kreipe, *Marrow fibrosis and its relevance during imatinib treatment of chronic myeloid leukemia.* Leukemia, 2007. **21**(12): p. 2420-7.

15. Hussein, K., O. Bock, A. Seegers, M. Flasshove, F. Henneke, G. Buesche, and H.H. Kreipe, *Myelofibrosis evolving during imatinib treatment of a chronic myeloproliferative disease with coexisting BCR-ABL translocation and JAK2V617F mutation.* Blood, 2007. **109**(9): p. 4106-7.

16. Arber, D.A., A. Orazi, R. Hasserjian, J. Thiele, M.J. Borowitz, M.M. Le Beau, C.D. Bloomfield, M. Cazzola, and J.W. Vardiman, *The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia.* Blood, 2016. **127**(20): p. 2391-405.

17. Tamascar, I. and J. Ramanarayanan, *Targeted treatment of chronic myeloid leukemia: role of imatinib.* Onco Targets Ther, 2009. **2**: p. 63-71.

18. Thiele, J., H.M. Kvasnicka, F. Facchetti, V. Franco, J. van der Walt, and A. Orazi, *European consensus on grading bone marrow fibrosis and assessment of cellularity.* Haematologica, 2005. **90**(8): p. 1128-32.

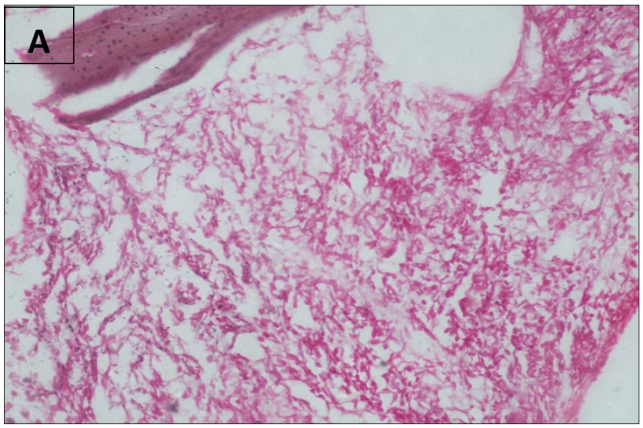
19. Buesche, G., R. Hehlmann, H. Hecker, H. Heimpel, B. Heinze, A. Schmeil, M. Pfirrmann, G. Gomez, A. Tobler, H. Herrmann, M. Kappler, J. Hasford, T. Buhr, H.H. Kreipe, and A. Georgii, *Marrow fibrosis, indicator of therapy failure in chronic myeloid*

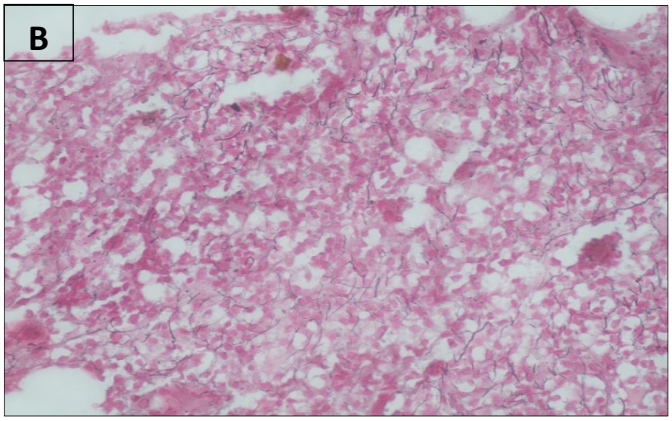
leukemia - prospective long-term results from a randomized-controlled trial. Leukemia, 2003. **17**(12): p. 2444-53.

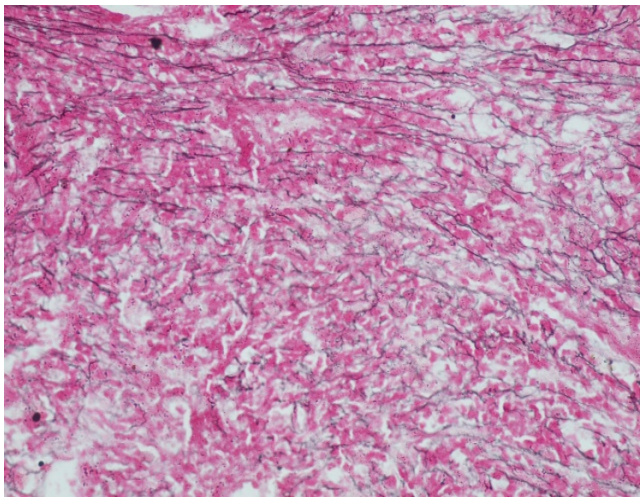
20. Vigna, E., B. Martino, F. Bacci, A.G. Recchia, F. Mendicino, R. Morelli, F.R. Mauro, C. Musolino, R. Greco, E. Lucia, E. Sabattini, F. Morabito, and M. Gentile, *Disappearance of Bone Marrow Fibrosis in a Patient with Chronic Myeloid Leukemia Treated with Dasatinib.* Chemotherapy, 2017. **62**(6): p. 350-352.
21. Le Bousse-Kerdiles, M.C., M.C. Martyre, and M. Samson, *Cellular and molecular mechanisms underlying bone marrow and liver fibrosis: a review.* Eur Cytokine Netw, 2008. **19**(2): p. 69-80.
22. Beham-Schmid, C., U. Apfelbeck, H. Sill, O. Tsybrovsky, G. Hofler, O.A. Haas, and W. Linkesch, *Treatment of chronic myelogenous leukemia with the tyrosine kinase inhibitor STI571 results in marked regression of bone marrow fibrosis.* Blood, 2002. **99**(1): p. 381-3.
23. McNamara, C., A. Grigg, J. Szer, A. Roberts, L. Campbell, R. Hoyt, K. Lynch, and S. Juneja, *Morphological effects of imatinib mesylate (STI571) on the bone marrow and blood of patients with Philadelphia chromosome (Ph) positive chronic myeloid leukaemia.* Clin Lab Haematol, 2003. **25**(2): p. 119-25.
24. Jesus, C., L. Ching, T. Neiva, and C. Vituri, *Assessment of fibrosis and vascularization of bone marrow stroma of Chronic Myeloid Leukemia patients treated with imatinib mesylate and their relationship with the cytogenetic response.* Brazilian Journal of Pharmaceutical Sciences 2011. **47**(2): p. 313-322.
25. Klion, A.D., J. Robyn, C. Akin, P. Noel, M. Brown, M. Law, D.D. Metcalfe, C. Dunbar, and T.B. Nutman, *Molecular remission and reversal of myelofibrosis in response to*

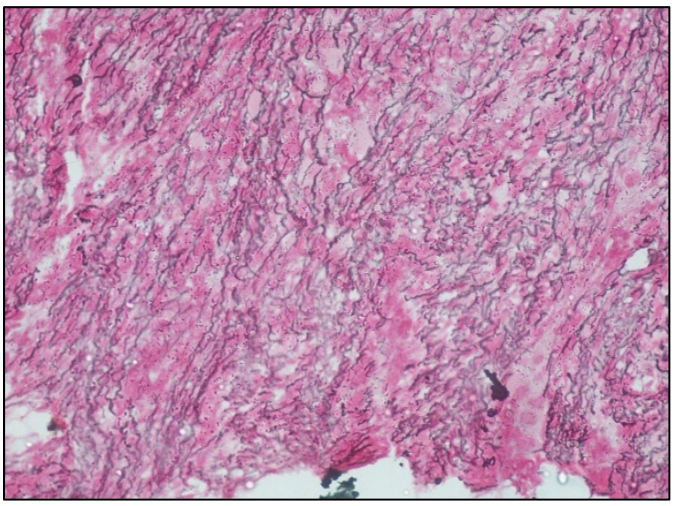
imatinib mesylate treatment in patients with the myeloproliferative variant of hypereosinophilic syndrome. Blood, 2004. **103**(2): p. 473-8.

List of figures:









C

D

Figure (1): Grading of bone marrow fibrosis (panel (A): MF-0: no fibrosis – panel (B): MF-1: loose network of reticulin fibers with many intersections especially in the perivascular area; panel (C): MF-2: diffuse and dense increase in reticulin fibers with extensive intersections, and occasionally focal bundles of collagen and panel (D): MF-3: dense increase in reticulin fibers with extensive intersections and coarse bundles of collagen).

List of tables:

Table (1): Demographic and clinical characteristics of CML patients:				
Total no of patients= 46				
	At presentation		**After one year of treatment**	
1. Age:				
Median:	44 years		45 years	
Range:	20:60 years.		20:60 years.	
2. Sex:				
Male:	22		47.8%	
Female:	24		52.2%	
3. Spleen size:				
No splenomegaly:	0	0%	10	21.7%
Mild splenomegaly:	0	0%	8	17.4%
Moderate splenomegaly:	18	39.1%	16	34.8%

Huge splenomegaly:	28	60.9%	12	26.1%
4. Hematological data:				
WBC (x 10^9/L)	166.8 ± 125.7		77.4 ± 99.3	
Hemoglobin (g/dL)	9.9 ± 1.2		10 ± 2.2	
Platelets (x 10^9/L)	378.5 ± 273.7		287 ± 276.8	
5. CML phase:				
CP	46	100%	38	82.6%
AP	0	0	2	4.3%
BP	0	0	6	13.1%
6. Sokal score:				
Low risk	10	21.8%		
Intermediate risk	18	39.1%		
High risk	18	39.1%		
7. Hasford score:				
Low risk	10	21.8%		
Intermediate risk	26	56.4%		
High risk:	10	21.8%		
8. EUTOS:				
Low risk	38	82.6%		
High risk	8	17.4%		
9. Hematologic response:				
Complete response:			18	39.1%
Partial response			28	60.9%
10. Cytogenetic response:				
Complete response:			16	34.8%
Major response:			2	4.3%
Partial response:			20	43.5%
No response:			8	17.4%
11. Molecular response				
Complete response:			16	34.8%
Major response:			30	65.2%

WBC: *White blood cell;* ***EUTOS:*** *European Treatment Outcome Study;* ***CML:*** *chronic myeloid leukemia;* ***CP:*** *Chronic phase;* ***AP:*** *Accelerated phase;* ***BP:*** *Blast phase*

Table (2): Assessment of bone marrow (BM) fibrosis				
Total no of patients: 46				
Grading	**BM fibrosis at presentation**		**BM fibrosis after treatment**	
	No.	**Percent (%)**	**No.**	**Percent (%)**
MF 0	14	30.4%	10	21.7%
MF 1	24	52.2%	8	17.4%
MF 2	8	17.4%	14	30.45%

MF 3	0	0%	14	30.45%
Total no with BM fibrosis	**32**	**69.6%**	**36**	**78.3%**

Table (3): Relation between BM fibrosis and clinical & hematological characteristics of CML patients:									
Clinical and hematological data	**At presentation**				**After one year of treatment**				
	Mean ± SD			**P Value***	**Mean ± SD**				**P Value***
	MF-0	**MF-1**	**MF-2**		**MF-0**	**MF-1**	**MF-2**	**MF-3**	
Age (Years)	28.9 ± 8.8	25.3 ± 11.6	41.8 ± 10.3	**<0.001**	30 ± 10.2	31.9 ± 6.8	46.6 ± 9	47.3 ± 12.7	**<0.001**
WBC count ($x10^9$/L)	136.5 ± 89.7	179.4 ± 151.8	182.3 ± 90.7	0.566	43.2 ± 45.02	39.9 ± 50.5	84.3 ± 111.6	116.4 ± 124.4	0.21
Hb level (g/dL)	9.9 ± 0.9	9.8 ± 1.3	10.6 ± 1.3	0.321	11.4 ± 2.1	9.2 ± 1.4	10.5 ± 2.3	9.1 ± 2.2	**0.039**
Platelet count ($x10^9$ /L)	284.9 ± 216.9	411.7 ± 289.9	442.8 ± 303.3	0.303	231.6 ±120.5	186.9 ± 122.5	290 ± 202.8	380.8 ± 433.5	0.393
PB Blast count (%)	3.2 ± 1.8	3.5 ± 2.4	4.5 ± 2.2	0.431	0.9 ± 1.3	2.9 ± 7	2.6 ± 3.3	2.6 ± 5.04	0.73
PB Basophils (%)	4.6 ± 3.1	4.8 ± 2.9	5.4 ± 2.4	0.676	1.6 ± 0.84	1.4 ± 0.52	6 ± 8.9	6.8 ± 8.9	0.162
BM Blast count (%)	6.3 ± 5.01	5.1 ± 2.3	5.5 ± 1.9	0.562	3.7 ± 2.2	5.9 ± 11.9	8.1 ± 12.3	14.1 ± 23.3	0.398
BM Basophils (%)	5.7 ± 4.03	4.6 ± 2.4	3.3 ± 2.1	0.218	1.2 ± 0.4	1 ± 0.00	6.4 ± 11.1	6.9 ± 10.9	0.226
BM Lymphocytes (%)	4.9 ± 2.03	6.6 ± 4.7	4 ± 1.9	0.159	14.8 ± 10.2	16.3 ± 9.3	13.2 ± 11.4	11.3 ± 8.4	0.687

***Oneway ANOVA (*P*<0.05 is significant)**

WBC: *White blood cell;* ***Hb:*** *Hemoglobin;* ***PB:*** *Peripheral blood;* ***BM:*** *Bone marrow;* ***Ph:*** *Philadelphia;* ***BCR:*** *Breakpoint Cluster Region;* ***ABL:*** *Abelson Leukemia Virus gene;* ***SD:*** *Standard deviation.*

Table (4): Relation between BM fibrosis at presentation and prognostic factors of CML patients:										
Total no.= 46				**MF 0**		**MF 1**		**MF 2**		**P value***
				No	**%**	**No**	**%**	**No**	**%**	
Sokal score	**Low risk**	10	21.7%	6	13.04%	4	8.7%	0	0%	**0.047**
	Intermediate risk	18	39.15%	4	8.7%	12	26.1%	2	4.35%	
	High risk	18	39.15%	4	8.7%	8	17.4%	6	13.04%	
Hasford score	**Low risk**	10	21.75%	2	4.35%	6	13.1%	2	4.35%	0.085
	Intermediate risk	26	56.5%	12	26.1%	10	21.7%	4	8.7%	
	High risk	10	21.75%	0	0%	8	17.4%	2	4.35 %	
EUTOS	**Low risk**	38	82.6%	12	26.1%	20	43.5%	6	13.04%	0.809
	High risk	8	17.4%	2	4.35%	4	8.8%	2	4.35%	

***Pearson Chi-Square (*P*< 0.05 is significant)**

EUTOS: *European Treatment Outcome Study.*

Table (5): Relation between BM fibrosis after treatment and response of CML patients:										
Total no.= 46		**MF 0**		**MF 1**		**MF 2**		**MF 3**		**P value***
		No	**%**	**No**	**%**	**No**	**%**	**No**	**%**	
Clinical response	**Complete**	5	10.9%	5	10.9%	4	8.69%	4	8.69%	0.312
	Partial	5	10.9%	3	6.5%	10	21.7%	10	21.7%	
Cytogenetic response	**Complete**	5	10.9%	3	6.5%	4	8.69%	4	8.69%	0.413
	Major	0	0%	0	0%	1	2.2%	1	2.2%	
	Partial	5	10.9%	5	10.9%	4	8.69%	6	13%	
	No	0	0%	0	0%	5	10.9%	3	6.5%	
Molecular response	**Complete**	5	10.9%	3	6.5%	4	8.69%	4	8.69%	0.681
	Major	5	10.9%	5	10.9%	10	21.7%	10	21.7%	

***Pearson Chi-Square (*P*< 0.05 is significant)**

Table (6): Relation between grades of BM fibrosis at presentation and after treatment					
Total no of patients: 46					
Grading	**BM fibrosis at presentation**		**BM fibrosis after treatment**		**P value***
	No.	**Percent (%)**	**No.**	**Percent (%)**	
MF 0	14	30.4%	10	21.7%	0.432
MF 1	24	52.2%	8	17.4%	**< 0.001**
MF 2	8	17.4%	14	30.45%	0.143
MF 3	0	0%	14	30.45%	**< 0.001**

*** Pearson Chi-Square (*P*< 0.05 is significant)**