



## TO EVALUATE THE SIGNIFICANCE OF RETICULIN STAIN MEASURED FIBROSIS IN CHRONIC MYELOGENOUS LEUKEMIA

### Histopathology

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### ABSTRACT

Chronic myelogenous leukemia is a clonal, myeloproliferative disease that develops when a single, pluripotent, haemopoietic stem cell acquires the Philadelphia chromosome, is frequently associated with myelofibrosis. This prospective study was conducted in the department of pathology, GMC Amritsar. All diagnosed patients of chronic myelogenous leukemia based on PBF and bone marrow study were included in the study. However, patients of essential thrombocythemia, polycythemia vera and myelofibrosis were not included in study. Bone marrow biopsies were performed on 25 cases of CML (newly diagnosed cases and patients on treatment for CML). Cut sections from bone marrow biopsies was stained with reticulin stain and then studied for fibrosis and graded. Some degree of fibrosis was noticed in all the patients of CML. In newly diagnosed cases of CML, most of patients show grade 1 and 2 fibrosis in chronic phase. Patient presenting in accelerated phase and blast phase of cml show grade 3 and 4. Patients of CML responding to treatment mostly show grade 1 and few showed grade 2. However, patients not responding to treatment has higher grade of fibrosis usually grade 3 or 4. Hence myelofibrosis is the indicator for poor prognosis of patients of cml and their responsiveness to treatment.

### KEYWORDS

Chronic myelogenous leukemia, Bone marrow fibrosis, reticulin stain

### INTRODUCTION

Chronic myelogenous leukaemia (CML) is a clonal, myeloproliferative disease that develops when a single, pluripotent, haemopoietic stem cell acquires the Philadelphia chromosome, is frequently associated with myelofibrosis. The haemopoietic cell lines are transformed by the chimeric oncogene BCR-ABL. CML has a triphasic or biphasic clinical course: a chronic phase, an accelerated phase, and blast crisis. However, about 20% to 40% of patients die of complications of marrow failure with associated fibrosis.<sup>1</sup>

Bone marrow fibrosis is a central pathological feature and major diagnostic criterion of myelofibrosis. Although bone marrow fibrosis is seen in a variety of malignant and non-malignant disease states, the deposition of reticulin and collagen fibrosis in the bone marrow of patients with chronic myelogenous leukemia is believed to be mediated by the myelofibrosis hematopoietic stem/progenitor cell. Increased expression of inflammatory cytokines, lysyl oxidase, transforming growth factor- $\beta$ , impaired megakaryocyte function, and aberrant JAK-STAT signaling have all been implicated in the pathogenesis of bone marrow fibrosis.<sup>2</sup>

Reticulin was initially described in 1892 as the powdery material between collagen fibers and is identified by various silver staining methods (reticulin stain).<sup>3</sup>

### OBJECTIVES

- To study the role of reticulin stain in patients with chronic myelogenous leukemia.
- To study the measured fibrosis with grading of reticulin stain.

### MATERIALS AND METHODS

This prospective study was conducted in the department of pathology, GMC Amritsar from April 2021 to December 2022. After approval from institutional ethical committee a written and informed consent was taken along with relevant history and bio data of the patients. All

diagnosed patients of chronic myelogenous leukemia based on PBF and bone marrow study were included in this study. However, Patients of primary myelofibrosis, essential thrombocythemia and polycythemia vera were excluded from study.

Bone marrow biopsies were done from posterior iliac crest on 25 patients of CML out of which 14 were newly diagnosed and 11 were already on treatment. The trephine biopsy was processed in standardised manner and stained with hematoxylin and eosin. Reticulin staining was done on trephine biopsy to assess the degree of fibrosis. Grading of fibrosis was done according to modified Bauermeister classification. Data was recorded in a Microsoft excel spread sheet and analysed using statistical Package for IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp., Chicago. Qualitative variables, i.e cytopenias/ cytosis (anemia, thrombocytosis and leukocytosis), splenomegaly, hepatomegaly and lymph nodes were analysed to determine their association with grades of fibrosis using descriptive statistics and crosstabs, keeping the confidence interval of 95% and p-value of <0.05.

### RESULTS

A total of 25 cases were included in the study 14 out of which were newly diagnosed and 11 were already on treatment. Out these 16(64%) were males and 9(36%) were females. Majority of the patients were in age group of 31-40 years. Fibrosis of varying degree was seen in all patients. Grade 1 was seen in 2 (24%) patients, grade 2 in 8 (32%) patients, grade 3 in 5 (20%) patients and grade 4 was present in 6(24%).

CBC counts were analysed to assess any association between anemia, thrombocytosis and leucocytosis with advanced fibrosis. There was no significant association between anemia and thrombocytosis with fibrosis (p value=0.075 and 0.06). TLC count was associated in newly diagnosed (p value=0.03) but not associated in already diagnosed on treatment cases (p value=0.06).

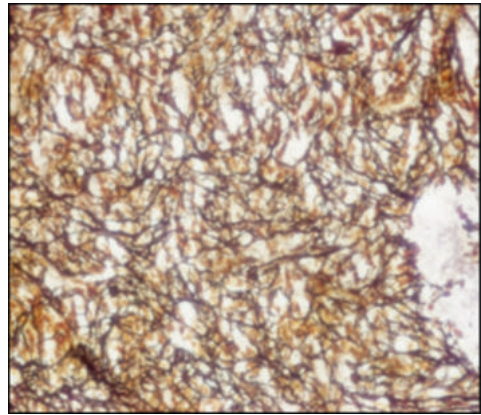
The correlations of reticulin fibrosis with various parameters are shown in Table 1 and 2. Significant (Grade 3 or 4) reticulin fibrosis was strongly associated with splenomegaly, increased marrow megakaryocytes and dysmegakaryopoiesis, and additional karyotypic abnormalities. Hepatomegaly did not show any correlation with the degree of marrow reticulin fibrosis.

**Table – 1 Correlation Between Splenomegaly And Hepatomegaly With Reticulin Fibrosis**

Reticulin Grade	Splenomegaly Present	Hepatomegaly Present
1	2	1
2	5	5
3	5	1
4	6	2
Total	18	9
p-value	0.043	0.07

**Table – 2 Correlation Between Megakaryocyte Count And Dysmegakaryopoiesis With Reticulin Fibrosis**

Reticulin Grade	Megakaryocytes Raised	Dysmegakaryopoiesis Present
1	2	1
2	4	4
3	4	4
4	6	6
Total	16	15



**Figure 4: Reticulin Stain Showing Grade 4 Fibrosis**

**DISCUSSIONS**

Chronic myelogenous leukemia is a clonal malignancy and is uncontrolled proliferation of myeloid series cells and megakaryocytes leading to bone marrow fibrosis and it represents a negative prognostic factor.<sup>4</sup> We conducted study on 25 cases of CML to study the effect of bone marrow fibrosis by reticulin staining on prognosis of the CML. Correlation between various prognostic factors like anaemia, cytosis (leucocytosis and thrombocytosis), organomegaly (splenomegaly, hepatomegaly), megakaryocytes count and dysmegakaryopoiesis with reticulin grade was also made in this study.

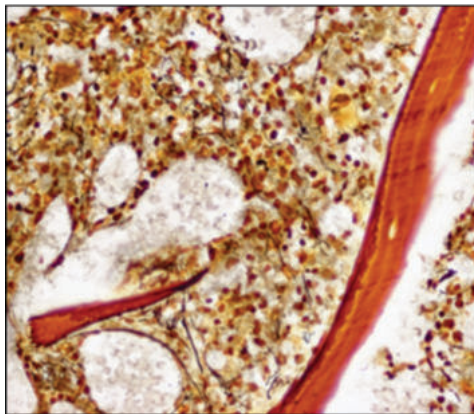
In present study, patients of all the age groups were included but peak was seen in the age group of 21-50 years in which peak was reported in the age group of 31-40 years. Dekmezian et al.<sup>5</sup> reported the peak age of 42 years in their study. In our study of 25 cases, majority of the cases i.e. 64% (n=16) were males. Females were in lower proportion 36% (n=9). Similar findings were also reported by Dekmezian et al.<sup>5</sup> We performed bone marrow aspiration on 25 cases which were hypercellular in all the 14 newly diagnosed cases and in 8 already diagnosed cases on treatment. Myeloid to erythroid ratio was also raised (>6:1) in all 14 newly diagnosed cases and 7 out of 11 already diagnosed on treatment cases. Chang F et al.<sup>6</sup> also reported similar findings.

Bone marrow biopsies were performed on 25 cases in our study, which were subjected to reticulin staining and grade of fibrosis was noted. It was reported that all the cases show some degree of fibrosis. Thirty two percent of cases has shown grade 2 fibrosis and while 24% of patients had grade 1 fibrosis. Significant fibrosis (grade 3 and 4) was seen in 44% (n=11) of patients. It was seen that 6 out of 11 patients with significant bone marrow fibrosis were newly diagnosed mainly shows high percentage of blast in bone marrow and other poor prognostic factors. Dekmezian R et al.<sup>5</sup> in accordance to our study, also reported poor prognosis factors in 65 (47%) newly diagnosed cases with significant Grade 3 or 4 reticulin fibrosis. Other 5 patients in our study showing significant fibrosis were already on treatment of CML showing poor response to treatment. Eliacik E et al.<sup>7</sup> and Kantarjian HM et al.<sup>8</sup> also reported similar findings.

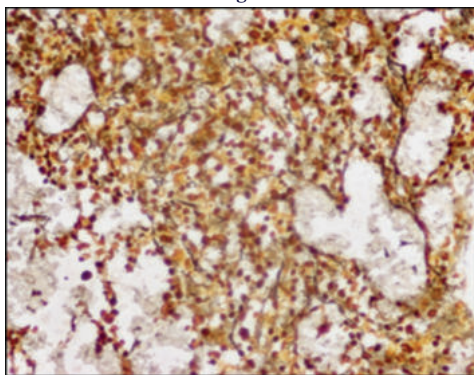
In our study correlation between other prognostic parameters such as anaemia, leucocytosis, thrombocytosis, and organomegaly with grade of fibrosis were also made. Anemia was not associated with advanced fibrosis. Hameed A et al.<sup>9</sup> also reported similar findings. In contrast, Saleem M et al.<sup>10</sup> found anaemia more with advanced fibrosis. TLC count was associated with fibrosis in newly diagnosed but no association was observed in already diagnosed on treatment cases. Failure of definitive conclusion in our study may due to the small study sample size in both the groups. In this study, platelet count was in normal range. The finding is supported by a study conducted by Cervantes F et al.<sup>11</sup>

In present study it was noted that Splenomegaly was present in all the cases with significant fibrosis versus half of cases in mild fibrosis and hence, correlated (p value =0.043). Findings in this study, are in accordance with Dekmezian R et al.<sup>5</sup> In present study, as hepatomegaly was present only in 9 cases and out of 25, no significant association of hepatomegaly was seen with grade of fibrosis. Saleem M et al.<sup>10</sup> also reported similar findings.

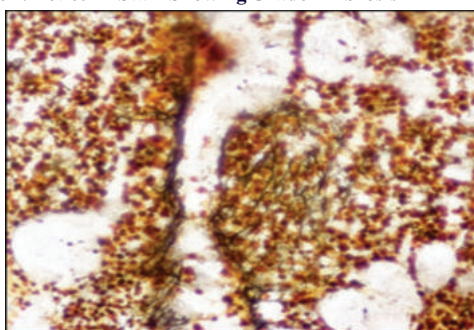
In our study, correlation between bone marrow megakaryocyte count



**Figure 1: Reticulin Stain Showing Grade 1 Fibrosis**



**Figure 2: Reticulin Stain Showing Grade 2 Fibrosis**



**Figure 3: Reticulin Stain Showing Grade 3 Fibrosis**

and fibrosis was also studied. It was observed that megakaryocytes were raised in 90% of patients showing higher grade of fibrosis as compared to 43,% with lower grade of fibrosis. This is in accordance to previous study performed by Khonglah Y et al.<sup>12</sup> and Buhr T et al.<sup>13</sup> In this study, association between dysmegakaryopoiesis in bone marrow specimens and grade of fibrosis were also made. 86% of patients with significant fibrosis has shown dymegakaryopoiesis. In accordance to our study Arunachalam AK et al.<sup>14</sup> also reported dyplastic features in megakaryocytes in all the patients with significant fibrosis.

## CONCLUSION

In present study, we observed an association between bone marrow fibrosis with poor prognostic factors of disease in newly diagnosed cases. It was also observed that already diagnosed cases showing poor response to treatment also showed significant fibrosis. All above data showed that myelofibrosis as an important prognostic indicator of CML.

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