

## Bone marrow trephine biopsy findings in chronic myeloid leukemia

Yookarin Khonglah MD, Debdatta Basu MD and \*Tarun Kumar Dutta MD

Departments of Pathology and \*Medicine, Jawaharlal Institute of Post Graduate Medical Education and Research, Pondicherry, India.

### Abstract

Sixty patients with chronic myeloid leukemia (CML) underwent bone marrow trephine biopsy at presentation. All the biopsies were decalcified, paraffin-embedded and stained with H&E, Gomori's reticulin and Masson Trichrome. A detailed study of the histology including the morphology and topographic distribution of megakaryocytes was done. 55 patients presented in chronic phase. Of these there were 37 cases (67%) of CML-granulocytic (CML-G) type and 18 cases (33%) of CML-granulocytic megakaryocytic (CML-GM) type. Five cases presented in blast crisis. 73% of CML-G had low-grade fibrosis while 83% of CML-GM had high-grade fibrosis. This was statistically significant. On follow-up 25% of CML-G went into blast crisis while all the CML-GM patients remained stable to date. Bone marrow biopsy is a useful investigation in patients of CML at diagnosis as it provides prognostic information. Evaluation of megakaryopoiesis, grading of fibrosis and localization of blasts are possible on a trephine biopsy.

**Key words:** Chronic myeloid leukemia, bone marrow trephine biopsy, myelofibrosis

### INTRODUCTION

The natural history of chronic myeloid leukemia (CML) is characterized by a chronic phase followed by a terminal blast crisis in most of the patients with a median survival of approximately 40 months.<sup>1</sup> The period of transformation is however variable and unpredictable.<sup>2</sup> There have been many attempts at identifying prognostic markers in CML.<sup>2,3</sup> Sokal *et al* evaluated the prognostic value of various clinical features of CML at diagnosis in what is now termed as the Sokal score.<sup>4</sup> Studies have lately focussed attention on the histopathology of bone marrow in providing valuable information.<sup>5-9</sup> A distinct clinicopathological subtype of CML was first recognized by Georgii *et al* based on bone marrow histology, which they labeled as chronic megakaryocytic-granulocytic myelosis.<sup>6</sup> Burkhardt *et al* proposed a working classification of chronic myeloproliferative disorders based on histological, haematological and clinical findings.<sup>7</sup> CML was subclassified into (i) granulocytic and (ii) granulocytic-megakaryocytic, depending on the morphology and distribution of the megakaryocytes in the bone marrow biopsy sections.<sup>9</sup> This histological classification of CML constituted an additional parameter in the diagnostic protocol of CML as it provided valuable information regarding the prognosis of individual patients.<sup>7,9,10</sup> A few

authors have however questioned the justification of this classification.<sup>11</sup>

The objective of this present study was to classify cases of CML into histological subtypes and to see if this classification had any bearing on the prognosis of the cases.

### MATERIALS AND METHODS

Bone marrow trephine biopsy along with aspiration was done from the posterior superior iliac spine in sixty patients of CML at diagnosis. The diagnosis of cases as CML was based on their clinical features, peripheral blood findings and leukocyte alkaline phosphatase score. Cytogenetic analysis for Philadelphia chromosome, however, could not be done. None of the patients had received any therapy at the time of the biopsy. The biopsy was fixed in buffered formalin for 18 hours and decalcified in 10% formic acid for 4 to 6 hours. Paraffin-embedded sections, cut at 4 microns were stained with Haematoxylin and Eosin. In each case, Gomori's reticulin stain and Masson Trichrome stain were also done.

Based on the criteria laid down by Bartl *et al*<sup>9</sup> cases were classified as CML granulocytic (CML-G) and CML granulocytic-megakaryocytic (CML-GM). A detailed study of the morphology and distribution of megakaryocytes was done as described by Burkhardt *et al*.<sup>7</sup> Megakaryocytes

were classified as normal, dwarf, immature, giant, pleomorphic, blastic or pyknotic. The distribution was noted as diffuse, sheet or clustered. Fibrosis was graded from Grade 1 to Grade 3B, grade 1 to 3A being reticulin fibrosis and grade 3B signifying laying down of collagen.<sup>12</sup> Aspiration smears were stained with Leishman's smear. The duration of survival till the last visit or the time of death was also noted. Statistical analysis was done by Chi square test with Yates correction.

## RESULTS

Of the 60 cases of CML, 55 were in the chronic phase while 5 cases presented in blast crisis.

The 55 cases in chronic phase were subdivided into 37 cases of CML-G (67%) and 18 cases of CML-GM (33%).

The bone marrow biopsy was hypercellular in all the 55 cases presenting in chronic phase, with a cellularity ranging from 85-100%. Erythropoiesis was suppressed in all but one case. In all cases granulocytic proliferation dominated (Fig 1). This consisted of broad paratrabecular and perivascular seams of immature myeloid cells and mature granulocytes seen in the centre of the marrow spaces. Eosinophilia was commonly present. In two patients there was focal increase in blasts and

one of them subsequently went into blast crisis. In 4 cases pseudo Gaucher cells and in two cases, sea blue histiocytes were identified. In 71% of cases of CML-G, megakaryopoiesis was normal or even increased; however, most of them were dwarf forms and present in a diffuse manner. The megakaryocytes were increased in all cases of CML-GM (Fig 2). The important feature seen in this group was the topographic disorganization of the megakaryocytes, which were present in sheets and clusters (Fig 3). In contrast to CML-G, the megakaryocytes in CML-GM, were mainly pleomorphic and giant (Fig 4).

In CML-G, 73% of cases had grades 1 and 2 fibrosis (Fig 5) in contrast to CML-GM where 83% cases had high-grade fibrosis, i.e. grade 3A and 3B (Fig 6). This was found to be statistically significant ( $p < 0.001$ ). A graph detailing the type of CML and the grade of fibrosis is presented in Figure 7. Osteomyeloid sclerosis was seen in one case.

Bone marrow aspiration was unsuccessful leading to dry taps in 5% of CML-G and in as many as 33% of CML-GM cases.

In the five cases that presented in blast crisis, bone marrow biopsy showed sheets of blasts with suppression of erythroid series. Megakaryopoiesis was suppressed in three and increased in two of these cases.

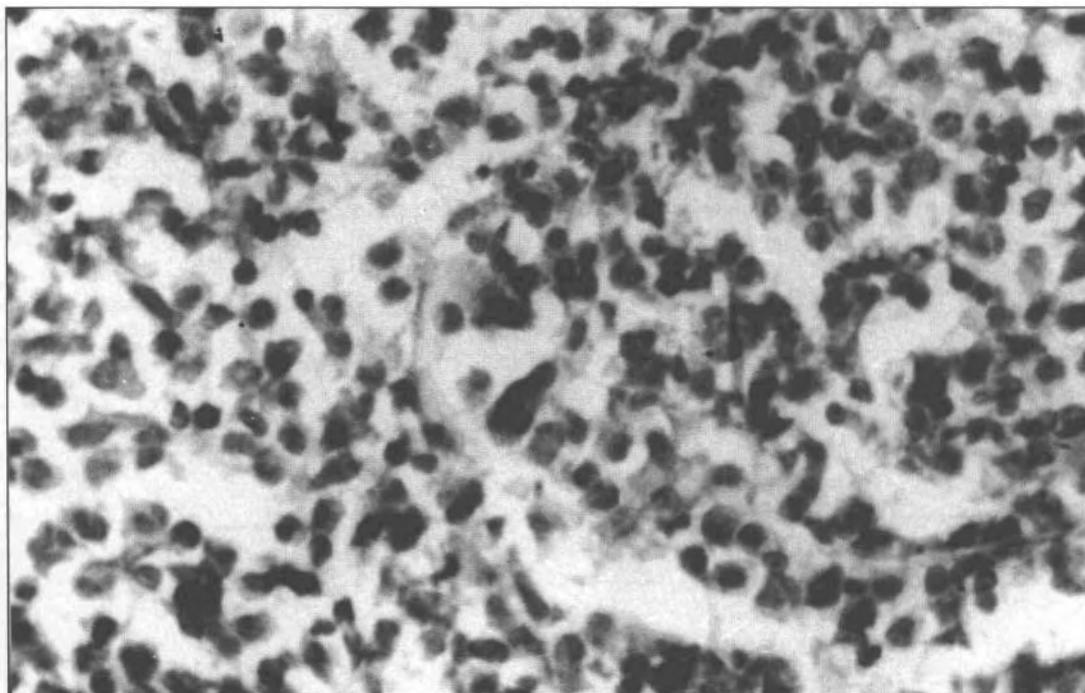


FIG. 1: Bone marrow biopsy showing florid granulocytic hyperplasia in a case of CML-G (H&E X 320)

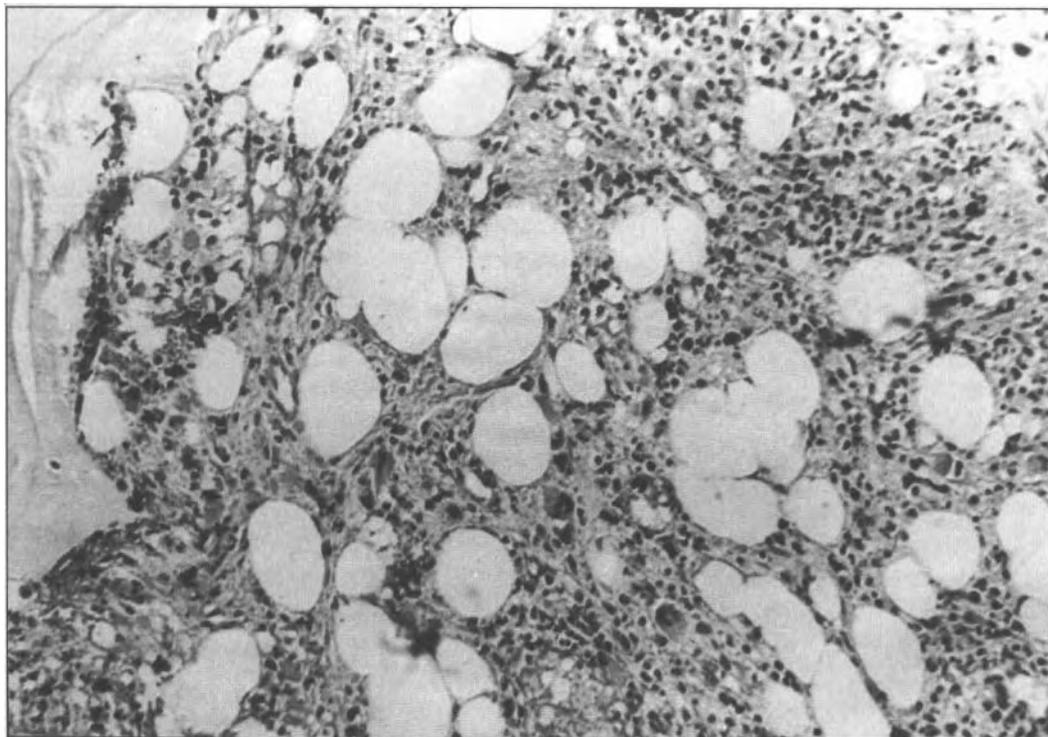


FIG. 2: Bone marrow biopsy in a case of CML-GM showing increase in number and clusters of megakaryocytes along with granulocytic hyperplasia (H&E X 160)

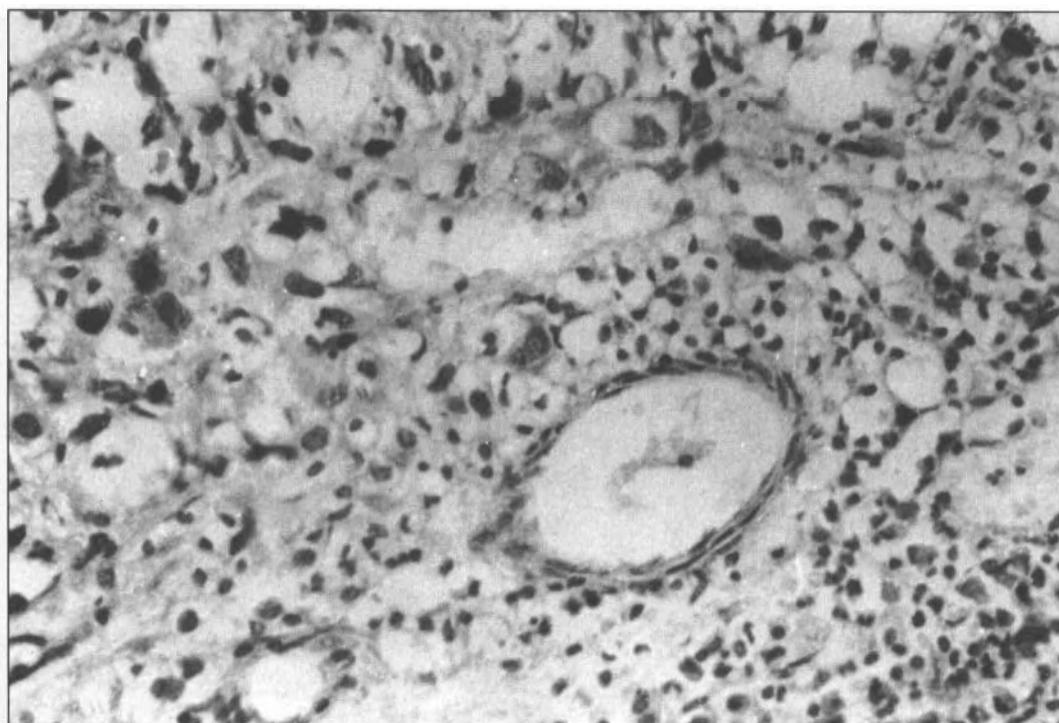


FIG. 3: Sheet of immature and pleomorphic megakaryocytes, showing topographic disorganization, in a case of CML-GM (H&E X 640)

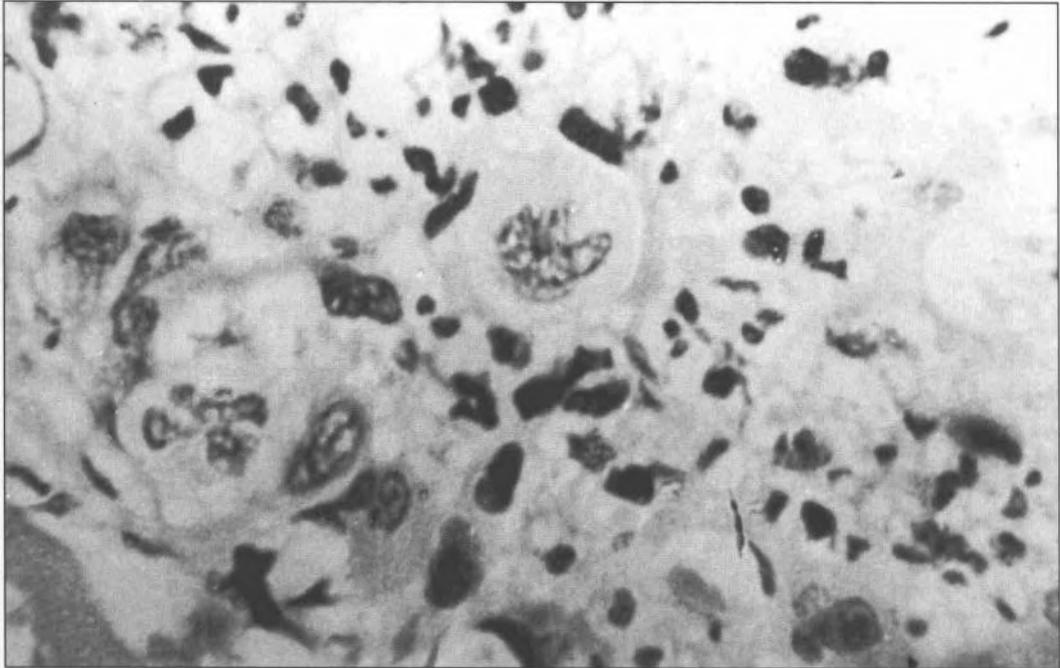


FIG. 4: Clustering of pleomorphic megakaryocytes in a case of CML-GM (H&E X 640)

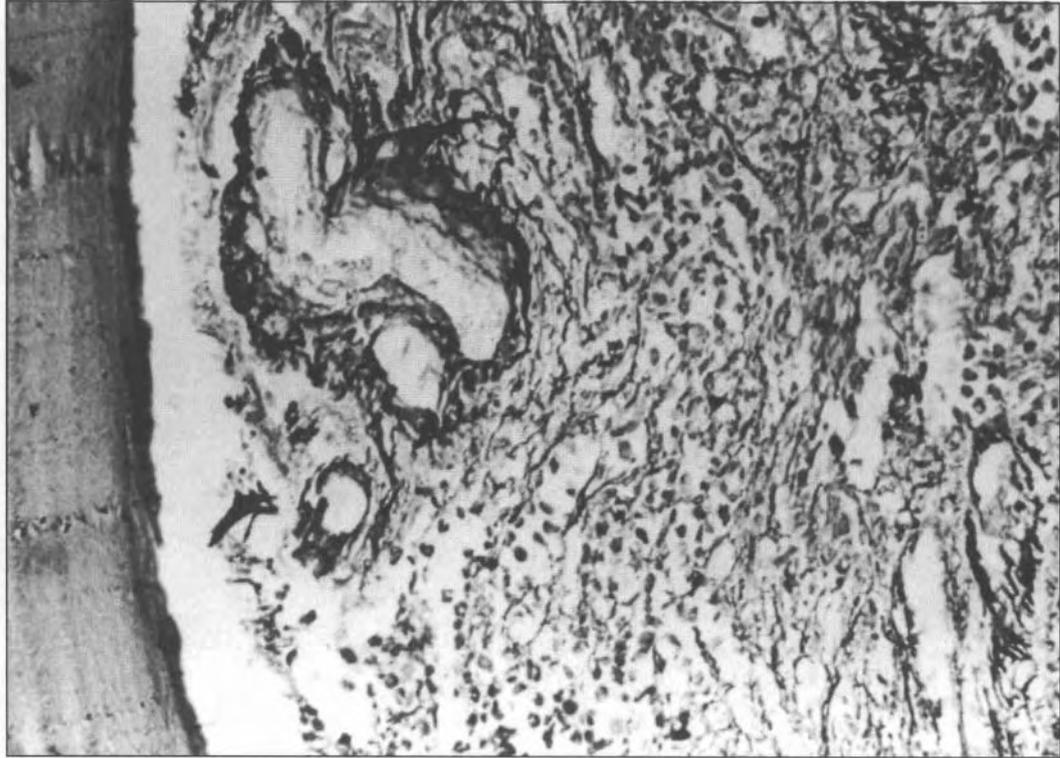


FIG. 5: Grade 1 fibrosis in a case of CML-G. Note that it is mainly perivascular (Gomori's reticulin X 160)

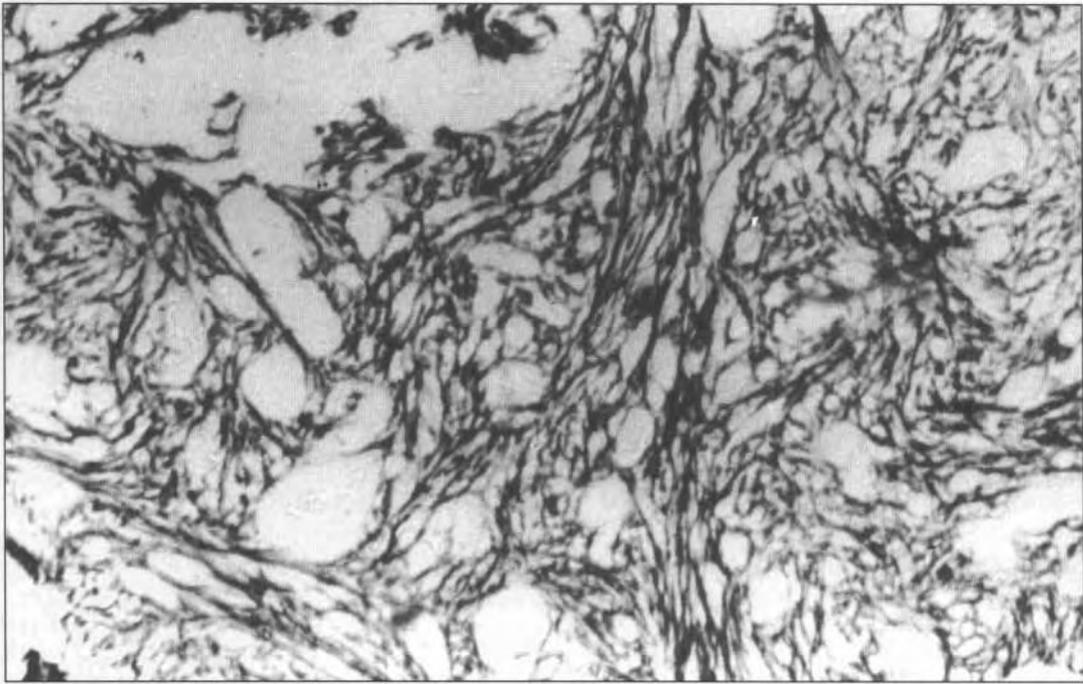


FIG. 6: Grade 3A fibrosis in a case of CML-GM showing marked increase in coarse reticulin fibers in marrow (Gomori's reticulin X 160)

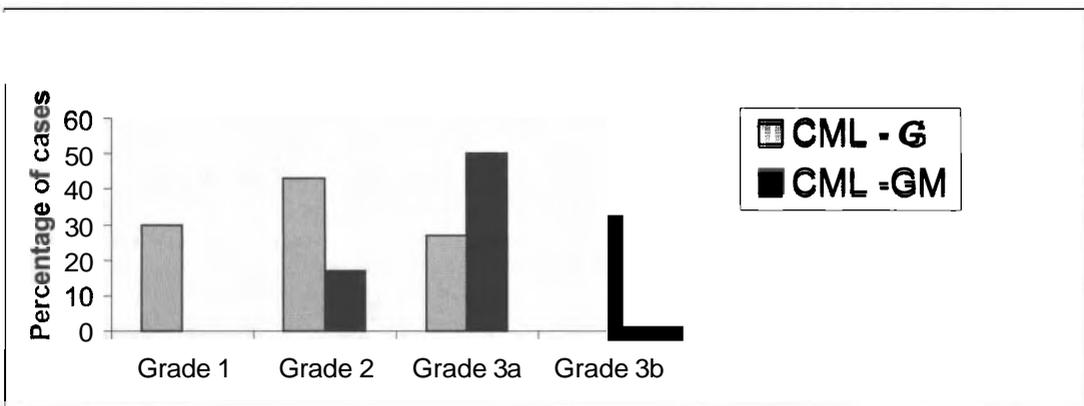


FIG. 7: Comparison of grade of fibrosis in the two histological subtypes of CML.

Forty-four of the sixty patients could be followed up, the duration of follow-up ranging from 3 to 30 months. Seven of the 28 cases of CML-G that have been followed have already progressed to blast crisis and five of them have expired. On the other hand, all the 16 cases of CML-GM are stable till date.

## DISCUSSION

Extensive work on the histology of chronic myeloproliferative disorders was done by Burkhardt *et al* and they identified two histological subtypes of CML, which behaved

differently during the course of the disease.<sup>7</sup>

Bartl *et al*<sup>9</sup> in their study of 1200 biopsies from 550 patients of CML classified the cases into:

- Unilinear granulocytic type (45%) which showed striking proliferation of granulocytes with low, normal or increased megakaryocytes which were typically dwarf forms, also called micromegakaryocytes.
- Bilinear granulocytic/megakaryocytic type (55%) where megakaryocytes were always increased and were highly pleomorphic with topographic disorganization and arranged in sheets and clusters.

Following these criteria, we found 67% cases of CML-G and 33% cases of CML-GM. This is in contrast to most other studies where CML-GM is more common, usually constituting 55% to 72% of cases.<sup>7,9,10,13</sup> Only Rozman *et al* found a higher number of cases of CML-G type." We believe that CML-G does not imply only granulocytic hyperplasia as was described initially by Burkhardt *et al*.<sup>7</sup> A good number of cases of CML-G showed an increase in number of micromegakaryocytes.<sup>9</sup> However they are not pleomorphic and did not show topographic disorganization. In our opinion only those cases that show pleomorphism and topographic alteration should be classified as CML-GM.

The need for such a detailed study of the type and distribution of megakaryocytes is emphasized as cases of CML-GM generally tend to fare better and remain in a chronic phase for a longer duration than those of CML-G who have a tendency to metamorphose early.<sup>7,10</sup> The median survival of patients with CML-GM was longer (28 months) than those with CML-G (21 months) in a study by Varma *et al*.<sup>13</sup> Our follow-up period of 30 months may not be adequate to study the metamorphosis of CML, as the period of transformation can be highly variable.<sup>1</sup> However, a definite trend was seen as 25% of cases of CML-G progressed to blast crisis in this period while all the cases of CML-GM remained stable. Burkhardt *et al* in their study of 149 patients found that 69% of cases of CML-G progressed to crisis in contrast to 21% of CML-GM.<sup>14</sup> Similarly Singh *et al* observed that 53% of CML-G developed blast crisis compared to 26% of CML-GM.<sup>10</sup> Although Varma *et al* did not find any difference in the frequency of blast crisis in the two groups, they did note that patients with CML-G tended to evolve into crisis much earlier. Within 12 months, 37% of patients with CML-G evolved into blast crisis in contrast to 15% of CML-GM.<sup>13</sup> The dwarf megakaryocytes, which are diffusely present characteristically in CML-G, have a tendency to develop into immature forms and may result in early onset of blast crisis.<sup>7</sup>

Majority of the cases of CML-G that we encountered showed low-grade fibrosis compared to CML-GM where 83% had high-grade fibrosis. This difference was statistically significant. The high incidence of fibrosis accounted for the higher rate of unsuccessful aspiration in CML-GM. Singh *et al* had found 96% cases of CML-G with grade 1-2 fibrosis while 50% of CML-GM had grade 3 fibrosis.<sup>10</sup> Varma *et al*,<sup>13</sup> however did not find any difference

in the degree of fibrosis between the two groups, which is in contrast to most other studies.<sup>7,9,10</sup> Mixed type of CML has a greater tendency to develop myelofibrosis and osteomyeloclerosis.<sup>7,9,14</sup> Patients with high-grade fibrosis have long and variable survival pattern and may not be necessarily associated with poor prognosis in terms of development of early blast crisis. However they do have problems related to marrow failure, especially during chemotherapy.<sup>5</sup> Development of myelofibrosis may decrease the onset of blast crisis and thus is associated with longer survival.<sup>14</sup> Many workers have however found myelofibrosis to be one of the poor prognostic indicators.<sup>3,12,15</sup> This contrasting feature reflects the variable and unpredictable nature of the disease and needs to be addressed. In a recent article, Kvasnicka *et al*, using multivariate analysis, reported that fibrosis was one of the poor prognostic features and that marrow histology must be included in the assessment of prognosis in all patients of CML.<sup>15</sup> The natural course of the disease involves progressive increase in marrow reticulin content.<sup>5,12</sup> Higher incidence of fibrosis has been noted in subsequent biopsies when compared with the initial biopsy taken at diagnosis.<sup>13</sup> The grade of myelofibrosis at clinical diagnosis of CML reflects the point in the evolution at which the disease is discovered. The number of marrow megakaryocytes appears to be the most important cytological variable that correlates with the degree of fibrosis.<sup>12</sup> Interactions between heterotopic megakaryocytes, interstitially deposited platelets, monocytes and lymphoid nodules induce the production of various growth factors e.g. platelet derived growth factor, epidermal growth factor, fibroblastic growth factor and others. All these induce fibroblastic proliferation and production of collagen.<sup>1</sup>

The present study included 5 (3%) patients who presented in blast crisis. Patients with CML, who present in blast crisis without the usual chronic phase, may represent those who were either symptom free or do not seek medical attention early.<sup>16</sup> The bone marrow in these cases showed diffuse infiltration by blasts in contrast to cases in chronic phase where the blasts are usually inconspicuous. In two of our cases presenting in chronic phase there was focal paratrabecular expansion of blasts. One of them progressed to blast crisis during the period of this study. Expanding paratrabecular seams of blasts herald metamorphosis of the chronic phase to blast crisis. Such cases need a close

follow-up with repeated biopsies.<sup>9</sup>

Bone marrow trephine biopsy allows subtyping of CML into two prognostically different groups. This is based on the morphology and distribution patterns of megakaryocytes. Grading of fibrosis is also possible in a biopsy. The degree of fibrosis differs considerably in the two groups. Another advantage of studying trephine biopsy at diagnosis is that cases with focal expansion of blasts especially in the paratrabeular region can be identified. Such patients need to be closely monitored, as they tend to metamorphose to blast crisis early. We recommend the use of a bone marrow trephine biopsy in the initial work-up of cases of CML.

## REFERENCES

1. Spiers ASD. Metamorphosis of chronic granulocytic leukemia. Diagnosis, classification and management. *Br J Haematol* 1979; 41:1-7.
2. Gomez GA, Sokal JE, Walsh D. Prognostic features at diagnosis of chronic myeloid leukemia. *Cancer* 1981; 47:2470-7.
3. Theologides A. Unfavourable signs in patients with chronic myeloid leukemia. *Ann Intern Med* 1972; 76:95-9.
4. Sokal JE, Cox EB, Baccarani M, Tura S, Gomez GA, Robertson JE *et al.* Prognostic discrimination in good risk chronic granulocytic leukemia. *Br J Haematol* 1984; 63:789-99.
5. Clough V, Geary GC, Hashmi K, Davson J, Knowlson T. Myelofibrosis in chronic granulocytic leukemia. *Br J Haematol* 1979; 42:515-26.
6. Georgii A, Vykoupil KF, Thiele J. Chronic granulocytic megakaryocytic myelosis (CMGM). A subtype of chronic myeloid leukemia. *Virchows Arch (Path Anat)* 1980; 389:253-68.
7. Burkhardt R, Bartl R, Jager K, Frisch B, Kettner G, Mahl G *et al.* Working classification of chronic myeloproliferative disorders based on histological, haematological and clinical findings. *J Clin Pathol* 1986; 39:237-252.
8. Cervantes F, Rozman C, Feliu E. Prognostic evaluation of initial histopathological features in chronic granulocytic leukemia. *Acta Haematol* 1989; 82:12-5.
9. Bartl R, Frisch B, Wilmanns W. Potential of bone marrow biopsy in chronic myeloproliferative disorders. *Eur J Haematol* 1993; 50:41-52.
10. Singh T, Bajaj R, Prakash S, Gaiha M. Evaluation of marrow histomorphology in prognosis of chronic myeloid leukemia. *Blood* 1990; 76(suppl): 321.
11. Rozman C, Cervantes F, Feliu E. Is the histological classification of chronic granulocytic leukemia justified from the clinical point of view? *Eur J Haematol* 1989; 42:150-4.
12. Lazzarino M, Morra E, Castello A, Inverardi D, Coco A, Pagnucco G *et al.* Myelofibrosis in chronic granulocytic leukemia: clinicopathological correlation and prognostic significance. *Br J Haematol* 1986; 64:227-40.
13. Varma N, Varma S, Marwaha N, Garewal G. Histological and clinical evolution patterns of chronic myelocytic leukemia. *Ind J Cancer* 1997; 34:164-8.
14. Burkhardt R, Jaeger K, Kettner G, Helmer G. Chronic myeloproliferative disorders: prognostic importance of new working classification. *J Clin Pathol* 1990; 43:357-64.
15. Kvasnicka HM, Thiele J, Schmitt-Graeff A, Diehl V, Zankovich R, Niederle N *et al.* Bone marrow features improve prognostic efficiency in multivariate risk classification of chronic phase Ph(1+) chronic myeloid leukemia: a multi center trial. *J Clin Oncol* 2001; 19:2994-3009.
16. Peterson LC, Bloomfield CD, Brunning RD. Blast crisis as an initial or terminal manifestation of chronic myeloid leukemia. *Am J Med* 1976; 60:209-220.