

HISTOLOGICAL CLASSIFICATION OF CHRONIC MYELOID LEUKAEMIA IN IRAQI PATIENTS

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Abstract

Background: Chronic myeloid leukemia (CML) is a stem cell disorder, which progresses from a "benign" chronic phase to a refractory acute leukemia. It is characterized by the presence of Philadelphia chromosome (Ph), in more than 90% of the cases.

Objective: To analyze comparatively histological and cytological features of bone marrow in CML at diagnosis, and to confirm the validity of histologic classification from the clinical point of view.

Methods: All cases were retrospectively collected from the Teaching Laboratories of the Medical City during the period of January 1985 and April 1994. Special attention was paid to histopathology of the bone marrow of diagnostic biopsies of 72 patients with chronic myeloid leukemia (CML) at the time of diagnosis prior to any therapy with particular reference to haemopoietic cellularity, megakaryocyte (MKCs) per unit area, reticulin fibrosis, blood vessels per unit area, osteoblast index, and trabecular bone width.

Results: Based on the number and morphological characteristics of MKCs, cases were classified into 31 patients with common type-chronic myeloid leukemia (CT-CML), and 41 with increased megakaryocytes (MI-CML). Both groups showed relevant clinical, haematological and histologic differences between them. The MI-CML was characterized by mixed proliferation of neutrophile, eosinophile and basophile series besides the megakaryocytic component, while the CT-CML cases predominantly revealed neutrophilic proliferation.

Conclusion: The MI-CML accumulated cases with unfavorable prognostic criteria such as older age, larger spleen, anemia, leucocytosis, thrombocytosis, higher percentage of normoblast, basophile and immature precursors (blast and promyelocyte). Among the quantitative parameters obtained from the bone marrow biopsies the quantitative fibrous tissue was significantly higher in MI-CML cases.

Key words: Chronic Myeloid leukemia, Iraqi Patients, Histological Classification.

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Introduction

Chronic myeloid leukemia (CML) is a stem cell disorder. Its natural history is characterized by a bi or tri-phasic courses^[1] and the presence of Philadelphia chromosome (Ph), which is a reciprocal balanced translocation of genetic material between the distal long arms of chromosomes 9 and 22 t (9; 22)^[2], resulting in a chimeric gene with a protein product has unique properties among other myeloproliferative disorders^[3].

Bone marrow trephine studies in CML at diagnosis have attracted the interest of a number of investigators. The bone marrow is markedly hypercellular, and haemopoietic tissue takes up to 70 - 90% of the marrow volume and fat is being reduced markedly^[4-7].

Megakaryocytes (MKCs) are normal or increased in number and occasionally clustered in groups of three or more in central intertrabecular regions^[8-12]. Megakaryocytes of CML are slightly smaller than normal (dwarf or micromegakaryocytes) with hypolobulated small nuclei and compact chromatin pattern and a relatively reduced rim of cytoplasm^[10].

Some cases of CML present with decreased number of MKCs^[11], and some authors proposed a subdivision of CML based on the number of MKCs^[11-13]; common type (CT-CML), has a decreased, normal, or slightly elevated number of MKCs, whereas a marked increase in MKCs may be called megakaryocytic CML or (MI-CML: chronic myeloid leukemia with

increased MKCs). A clinical significance to this division has not been demonstrated^[13].

In CT-CML type the hyperplasia affects the granulocytic cell line only, and erythropoiesis is reduced. Reticulin fibers were normal or only slightly increased, but fat cells were markedly reduced^[14,15]. The MI-CML is characterized by quantitative and qualitative alteration of megakaryocytopoiesis, as shown by occurrence of highly polymorphic, heterotopic, as well as immature megakaryocytes, the MKCs whether single or in clusters were usually situated in the central intertrabecular areas and close to hyperplastic sinusoids, frequently in their lumen^[14]. In some cases numerous small and micromegakaryocytes are present while in others coexistence of microform and giant cells, as well hypo- and hyperlobated nuclei, in addition to the pyknotic cells might be present^[13].

Materials and Methods

Records and materials of from January 1985 - April 1994 of 72 patients with chronic myeloid leukemia (non-blast phase) were revised, 39 patients were male and 33 were female. All cases were collected from Teaching Laboratories of the Medical City. The following variables are estimated in each case:

Clinical features: include, patient's age, liver and spleen size in centimeters below the left costal margin, and pre-diagnostic symptoms duration.

Haematological parameters: include, hemoglobin concentration (Hb g/dl), total leucocyte counts (WBC $\times 10^9/l$) and differential counts, and platelet counts (plt $\times 10^9/l$).

Bone marrow aspirate: Adequate aspirated material were available in 42 cases, and the following parameters were estimated; myeloid: erythroid ratio, and the percentage of basophils, eosinophils, promyelocytes, and blast cells.

Megakaryocytes were given the following score^[13]: (-) Totally absent, (+) Reduced,

(++) Normal, (+++) Slightly increased, and (++++ Moderately-Markedly increased.

Marrow trephine biopsy

Biopsy technique and evaluation: All the biopsies were cores obtained from posterior iliac crests and processed in standard technique. The minimal size of the cores was 2 x 12 mm. Two sections were obtained each of 5 μ m thickness, one stained with haematoxylin and eosin (H.&E.), and the other stained with Gomori's silver stain for reticulin fibers.

Cellularity (haemopoietic tissue)^[16]:

The percentage area of marrow occupied by haemopoietic tissue, fat, and fibrous tissue (H&E, and reticulin stain) was measured by "Chalkley point array graticule.

MKCs number and Blood vessels: were calculated by using a window with a known surface area inserted in the eyepiece of the microscope^[17].

Osteoblast index and Average trabecular bone width: were measured by special graticule (7x) calibrated at each magnification of the microscope^[15,18].

Statistical Methods: t test was used for comparing the mean values of the clinical, haematological, and histological parameters.

Results

On the basis of histologic assessment of the trephine, the cases were divided into two groups: CT-CML (common type CML), and MI-CML (CML with increased megakaryocytes)^[6-10,13,17,18]. CT-CML (31 cases): had predominant granulocytic hyperplasia, with normal or only slightly increase MKCs (MKC $\leq 42/mm^2$).

MI-CML (41 case): had a mixed granulocytic and megakaryocytic proliferation, (MKC $> 42/mm^2$). All the parameters analyzed in this study were compared between the two groups.

Clinical features:

Patients with MI-CML, were older age and had larger spleen, also they had

larger liver, and longer pre-diagnostic symptom duration. The last two variables failed to reach a significant level with statistical analysis.

Haematologic parameters:

The mean values of WBC, platelet counts, percentages of basophils, immature cells (promyelocytes and blasts), and circulating normoblasts were significantly higher in those cases with MI-CML. The same group had lower haemoglobin concentration, while the cases with CT-CML had significantly higher percentage of mature neutrophil.

Bone marrow aspirate features

(Tables 1, and 2): Cases with MI-CML had higher percentages of eosinophil and basophil, and higher M: E ratio than

cases with CT-CML. Bone marrow aspirates were also useful in distinguishing cases of CT-CML from MI-CML based on the number of MKCs. As can be seen in (Table II) CT-CML group had 17 bone marrow aspirates, 10 cases of them had normal MKCs (++) ,and 7 cases were slightly increase (+++). While in MI-CML there were 25 aspirates, 5 cases show slight increase in MKCs, while the other 20 had moderate-marked increase MKCs (++++), and there was statistically significant difference in MKC number in the aspirates of CT-CML, and MI-CML, as P-value was < 0.001. The same Table demonstrates significant correlation between the number of MKCs in aspirates and biopsies.

Table 1: Bone marrow aspirates findings of CT-CML and MI-CML.

Bone marrow aspirate	CT- CML.(19)* Mean ± SD	MI-CML.(23) * Mean ± SD	P-value
Basophil %	2.74 ± 2.68	5.04 ± 2.51	.005**
Eosinophil %	2.16 ± 2.69	5.30 ± 2.84	.031**
Promyelocyte%	4.32 ± 2.31	4.78 ± 2.13	.327[NS]
Blast %	2.83 ± 2.15	3.05 ± 2.22	.739[NS]
M:E Ratio***	19.3 ± 6.9	23.7 ± 12.3	.036**

* Number of cases in each stage. ** Significant. *** Myeloid: erythroid ratio. [NS]: Not significant. CT-CML: Common type chronic myeloid leukemia. MI-CML: Chronic myeloid leukemia-megakaryocytes increased.

Table 2: Semiquantitative of MKCs in bone marrow aspirate of CT-CML and MI-CML and its correlation with subjective estimation of MKCs in bone marrow biopsy.

		Subjective MKC in bone marrow biopsy				Total
		CT-CML (17)		MI-CML (25)		
		normal	Slightly increase	moderately increase	Markedly increase	
MKC in bone marrow aspirate	++	8	2			10
	+++	1	6	2	3	12
	++++			8	12	20
Total		9	8	10	15	42

++ Normal , +++ Slightly increase , ++++ moderately – markedly increase. P – value < 0.001
 CT-CML: Common type chronic myeloid leukemia.
 MI-CML: Chronic myeloid leukemia – megakaryocytes increased.

Bone marrow biopsy parameters

(Table 3): The percentage of marrow area occupied by fibrous tissue, and the average trabecular bone width were significantly higher in cases with MI-CML, than those with CT-CML, while the quantitative cellularity was higher in CT-CML but failed to reach a significant level.

The frequency of MKC clustering was higher in MI-CML (10 cases vs. 1

case), and the P-value was < 0.001. Also, there were highly significant differences in the morphology of MKCs, as in CT-CML 21 cases had normal morphology, and 10 cases showing abnormalities including hyposegmentation of the nuclei, and dwarfism. In MI-CML only three cases present with normal morphology of MKC and 38 cases exhibited the abnormalities mentioned earlier, and P-value was <0.001.

Table 3: Bone marrow biopsy features of CT-CML and MI-CML.

	CT- CML.(31)* Mean ± SD	MI-CML.(41)* Mean ± SD	P-value
Q. Cellularity (%)	84.0 ± 14.3	75.4 ± 19.2	0.062[NS]
Q. Fatty tissue %	5.6 ± 8.3	3.9 ± 5.6	0.333[NS]
Q. fibrous tissue (H.&E.)	10.4 ± 11.6	20.68 ± 18.35	0.009**
Q. fibrous tissue (Reticulin stain) (%)	29.1 ± 23.7	59.0 ± 27.1	<0.001**
MKC/mm²			
Blood vessels /100mm²	26.1 ± 7.1	81.7 ± 38.8	<0.001**
Osteoblast Index	2970.5 ± 1291.0	3627.9 ± 1424.2	0.071[NS]
Average trabecular bone width (µm)	0.74 ± .19	0.79 ± .25	0.581[NS]
	73.8 ± 11.8	81.7 ± 12.0	0.013**

Q. : quantitative. * Number of cases in each stage. ** Significant. [NS]: Not significant. CT-CML: Common type chronic myeloid leukemia. MI-CML: Chronic myeloid leukemia – megakaryocytes increased.

Discussion

The results of present study indicate that it is possible to distinguish on histologic grounds two forms of CML according to criteria described in previous studies^[6-10,13,17,18]. There are relevant clinical and haematological differences observed between the two groups of the patients.

The group of patients who presented with megakaryocytic hyperplasia MI-CML was older, and had larger spleen. The older age of MI-CML group are only reported by (Georgii, 1979,1980)^[19,20], but not by others^[13,21], and larger spleen in the same group also reported by Rozman, 1989^[21], who reported also that MI-CML associated with larger liver which was not proven statistically significant in the present study.

The MI-CML group differs significantly from the CT-CML, in their haematological parameters. The first group had lower haemoglobin concentration, higher M: E ratio in their bone marrow aspirates (which may be related to suppressed erythropoiesis), these finding

agree with that reported by Lorand, 1987^[13], and Burkhardt, 1982^[18], but differ from what had been reported by Knox, 1984^[6], Rozman, 1989^[21], and Bartl, 1982^[14]. The platelet count was higher in MI-CML than that of CT-CML, and this agrees with the results obtained by Rozman 1989^[21], and Burkhardt, 1982^[18]. The leucocytes were higher in MI-CML, confirming the results obtained by Lorand 1987^[13], but differed from the finding of higher WBC counts in CT-CML group mentioned by Burkhardt 1982^[18], and also differ from the results of Rozman, 1989^[21], and Knox, 1984^[6], who mentioned that there was no significant differences between the two groups.

Basophil percentage is higher in MI-CML, in both bone marrow, and peripheral blood, while eosinophil percentage approaches higher level in bone marrow of MI-CML but not in their peripheral blood. So MI-CML could be characterized as a CML with mixed proliferation of eosinophil, basophil, and megakaryocyte besides the neutrophil proliferation while

the CT-CML show predominantly neutrophilic proliferation. This is similar to the result of Lorand, 1987^[13], Burkhardt, 1982^[18], and Rozman, 1989^[21]. Burkhardt reported a combination of eosinophilic and megakaryocytic proliferation seen in bone marrow sections but not association of the proliferation of all 3 cell lines in MI-CML. While Frisch, 1985^[22] mentioned the reverse as he reported more eosinophilia in CT-CML.

Immature granulocytes (blast and promyelocyte) were more in MI-CML, and this is similar with the results obtained by Lorand, 1987^[13], Razzarino, 1986^[17], and Rozman, 1989^[21]. But differ from what reported by Bartl, 1982^[14], that the blast percentage was higher in CT-CML. The bone marrow aspirate parameters analyzed also permitted a differentiation between the two types of CML by the number of MKCs, as in both aspirate and biopsy there is a continuous spectrum ranging from cases with few normal looking MKCs to cases with a considerable proliferation was found, so this study showed that the parameters obtained from both the aspirate and biopsy were complimentary in classification, but in previous studies the classification was based on the histologic features of the trephine sections. Lorand, 1987^[13], depend on cytological features of bone marrow obtained from the imprints in classifying CML not from the aspirate as in the present study.

MI-CML showed striking qualitative and quantitative alteration in MKCs which were highly polymorphic, and had higher frequency for clustering, associated with higher incidence of fibrosis, which may be related to higher MKCs counts especially those with atypical morphology, as there is a strong correlation between MKCs counts and development of myelofibrosis^[23]. In the present study, as in previous studies^[13,14], MI-CML seems to concentrate cases with known unfavorable prognostic factors, such as older age, larger spleen, higher leucocyte and platelet counts, more frequent basophils, higher immature granulocytes,

higher circulating erythroblast, higher MKCs, and more frequent increase in reticulin fibers. However follow up of the cases is justified to predict the reliable survival rate.

Conclusion

Two types of CML, CT-CML, and MI-CML can be identified by the aid of histologic assessment of the trephine biopsy. MI-CML cases characterized by mixed proliferation of neutrophil, eosinophil, and basophil series, beside the proliferation of MKCs. While CT-CML showed a predominantly neutrophilic proliferation. The results of this study confirm the validity of histologic classification from the clinical point of view. The type MI-CML accumulates cases with unfavorable prognostic factors.

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