

SUBJECTIVE AND QUANTITATIVE EVALUATION OF BONE MARROW TREPHINE BIOPSY IN IDIOPATHIC MYELOFIBROSIS

Ahlam Al-Mashhadani¹ FIBMS, Saad Sh. Mansoor² FRCPath, Falih S. Sarhan² FIBMS

Abstract

Background: Idiopathic Myelofibrosis (IMF) is one of the myeloproliferative disorders in which various degrees of bone marrow fibrosis constitute the cardinal pathogenetic criteria for the disease therefore the study of bone marrow trephine biopsy is a major step in diagnosis .

Objectives: Are reticulin and iron stain are essential for the diagnosis and staging of idiopathic myelofibrosis

Methods: Trephine biopsies of 30 patients with IMF were reevaluated and the paraffin blocks were further sectioned, stained for H & E and reticulin and Perl's reaction. The patients were classified into four groups according to Cologne criteria for which both subjective and quantitative evaluations of trephine biopsies were performed.

Results: Both qualitative and quantitative evaluation was performed on trephine biopsies.

The most consistent finding with progression of diseases was megakaryocytic and granulocytic proliferation with preponderance of megakaryocytes. The study also proves that most patients showed an increase in the number of hemopoietic cells, reticulin fibers, trabecular bone width, osteoblastic index, blood vessels, and a reduction in the iron stores.

Conclusion: A thorough assessment of bone marrow biopsy including adequate tissue sampling stained for reticulin and iron stain are essential for the diagnosis and staging of idiopathic myelofibrosis.

Keywords: Idiopathic myelofibrosis, trephine biopsy, subjective, quantitative

IRAQI J MED SCI, 2005; VOL. 4 (2): 214-219

Introduction

Idiopathic myelofibrosis is an interesting myeloproliferative disease, which presents in middle aged and elderly people with signs and symptoms of anemia, splenomegaly, bone pain and hypermetabolic state with a leucoerythroblastic blood picture, teardrop cells and varying degree of bone marrow fibrosis^[1,2].

The principal pathogenetic criteria are an abnormal megakaryocyte resulting from an abnormal neoplastic stem cell. The death of these abnormal megakaryocytes results in the production of mitogens that result in bone marrow fibrosis^[3-7].

The aspiration of bone marrow in IMF patient usually yields dry tap or unsatisfactory marrow is collected^[3,4,8]. Therefore, trephine biopsy is essential for diagnosis, which shows an increase in reticulin fiber density and thickness^[3,4,10].

The diagnosis and staging of IMF is based on the Cologne Criteria^[11,12] and because of the prognostic significance of the histopathological features of IMF, this study

¹Specialist in Hematology, Ministry of Health-Al-Noor Hospital, ²Dept. Pathology, college of Medicine, Al-Nahrain University.

Address correspondence to: Dr. Falih Salim Sarhan.

Received 22nd May 2005; Accepted 21st December 2005

is performed to assess the marrow trephine findings in different stages of the disease.

Material & Methods

Using paraffin blocks of 30 patients with IMF, processed from Sep.1998 to Sep. 2002, 18 patients were males and 12 were females. Three sections were obtained from the original paraffin blocks and stained for H &E, reticulum and Perl’s reaction. Trephine biopsies were re-evaluated both subjectively and quantitatively.

Subjective evaluation involves the systemic examination of H & E slides for

1. **Cellularity:** graded as follows^[13,14] decreased <35%, normal 35-49%, slight Increase 50-59%, moderate Increase 60-89%, marked Increase 90-100%
2. **Megakaryocytic concentration**^[13,14] decreased <3/mm², normal 3-5/mm², slight increase 6-8/mm², moderate increase 9-14/mm², marked increase ≥15/mm²
3. **Iron content:** after staining the slides for Perl’s reaction the results were graded as follows^[15], 0: No iron, 1: Minimal iron, , 2: Slight & Patchy, 3: Moderate & Diffused, 4:

Strong and extensively diffused, Grades 0-1 indicate iron deficiency.

4-Reticulin fibrosis was evaluated as in Ellis et al^[14].

The quantitative evaluation of bone marrow biopsies involved counting the number of megakaryocytes, and blood vessels in one cubic mm using a planimetric method^[16]. Using the Chalkley’s point counting method, the amount of hemopoietic tissue was measured as percentage^[9].

Both of the osteoblastic index and the trabecular bone width was measured using an ocular graticule^[17]. Bone marrow biopsies of 30 subjects with normal histology was chosen as a base line data.

Statistical analysis was done using one way ANOVA and t-test, with P value less than 0.05 was considered significant.

Results

The subjective evaluation of trephine biopsies showed that most patients examined have increased cellularity (Table 1).

Table 1: Subjective evaluation of cellularity

Cellularity	No.	Percentage (%)
Reduced	12	40
Normal	4	13.34
Slightly increased	6	20
Moderately increased	5	16.66
Markedly increased	3	10

Megakaryocytes are increased in 76.66% of cases, being markedly increased

in 16.66%. In 20% of cases, clustering of megakaryocytes was recognized (Table 2).

Table 2: Subjective evaluation of megakaryocytes

Megakaryocytes	Evaluation	No. of cases	Percentages (%)
Number	Reduced	4	13.34
	Normal	3	10
	Slight increase	1	36.66
	Moderate increase	7	23.34
	Marked increase	5	16.66
Distribution	Diffuse	21	70
	Cluster	6	20
	Sheets	3	10

Reticulin fibrosis was evident in all cases; however, it was marked in 22 patients (Table 3).

Table 3: Subjective evaluation of reticulin fibrosis

Reticulin fibrosis	Number	Percentage (%)
Slight increase	3	10
Moderate increase	5	16.66
Marked increase	22	73.34

One third of patients showed iron deficient erythropoiesis with six patients showed no demonstrable iron in stores (Table 4).

Table 4: Semi quantitative evaluation of iron pigment

Marrow iron grade	Number of cases	Percentage
0	6	20
+	4	13.34
++	14	46.66
+++	6	20

The results of quantitative evaluation of trephine biopsies in patients with IMF are shown in table 5, which also compares the results with that of control.

Table 5: Comparison between quantitative evaluation of IMF patients and controls

	Patient mean	Control mean	P value
Hemopoietic tissue	44.32	50.93	<0.01
Fatty tissue	5.99	49.01	<0.001
Megakaryocytes (MKC)	43.60	19.09	<0.001
Osteoblastic index (OBI)	0.66	0.35	<0.001
Blood vessels (BV)	4021	2613	<0.001
Trabecular bone width (TBW)	116	78	<0.001

A correlation between quantitative evaluation of bone marrow biopsy & the degree of fibrosis was obtained and is shown in table 6.

Table 6: Correlation between degree of reticulin fibrosis & quantitative evaluation of bone marrow biopsy

Reticulin fibrosis	No. (%)	Hemopoietic. Tissue	Fatty*** tissue	Fibrous tissue	TBW	MKC	OBI	BV
Slight increase	3(10)	92.68	3.81	7.32	80.03	35.62	0.612	3025.5
Moderate increase	5(16.34)	80.06	7.79	33.67	102.12	37.15	0.635	3712.3
Marked increase	22(73.66)	31.53	10.60	45.05	116.88	46.37	0.676	4101.08
p value		<0.01	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

The Cologne criteria was used in this study for staging of IMF which showed that 10% of patients were stage I, 15% stage II,

40% stage III and 35% stage IV. Bone marrow biopsy finding at different stage of IMF is shown in table 7.

Table 7: BMB finding at different stages of IMF (one way ANOVA)

	Stage I	Stage II	Stage III	Stage IV	P value
Hemopoietic tissue	92.68	80.06	49.31	41.10	<0.05
Fatty tissue	3.81	7.79	9.24	16.92	<0.05
Fibrous tissue	7.32	33.67	44.58	49.06	<0.05
TBW	80.03	102.12	116.88	119.35	<0.05
MKC	35.62	37.15	45.91	49.36	<0.001
OBI	0.612	0.635	0.676	0.789	<0.05
BV	3025.5	3712.3	4059.6	4300.8	<0.05

Discussion

A better understanding of the myeloproliferative process and prognosis of IMF patients can be obtained through study of how this disease is expressed in the bone marrow. In this study both subjective and quantitative evaluation of bone marrow trephine biopsies was performed correlating the findings with the amount of iron pigment, reticulin fibrosis and staging of the disease.

The majority of cases showed hemopoietic hypercellularity rather than hypocellularity, a result similar to that of Varki et al, 1983^[18]. Many of the megakaryocytes showed morphologic abnormalities as those described by other studies^[19,20]. These abnormalities include bizarre nuclear configuration, most of the cells show hypo segmented nuclei while few megakaryocytes have hyper segmented nuclei^[19,20].

A significant degree of marrow fibrosis was recognized in most patients (73.34%) which is higher than that reported earlier by Varki et al, 1983^[18], but lower than the figure reported by Jalal (1988) who found that all cases showed marked degree of fibrosis^[21].

The semi quantitative evaluation of marrow iron stores revealed the presence of depleted iron stores in 33.34% of patients, presumably because of secondary iron deficiency due to blood loss either resulting from the presence of extramedullary hemopoietic foci leading to peptic ulcer^[22]

or due to platelet dysfunction, acquired factor V deficiency, thrombocytopenia and DIC^[23].

The quantitative evaluation of bone marrow biopsies showed that IMF patients have lower mean values of hemopoietic tissue and fatty tissue and by higher mean values of fibrous tissue; trabecular bone width, OBI, MKC concentration, and blood vessels (p value < 0.001). Similar findings were described by Frisch et al, (1985), who also described the correlation between the degree of reticulin fibrosis and quantitative evaluation of BMB^[17]. He proved that the mean value of hemopoietic tissue volume is reduced with increasing amount of reticulin fibrosis, while the mean values of fatty tissue volume, fibrous tissue volume, MKC concentration, OBI, TBW & blood vessels tend to correlate with the degree and reticulin fibrosis^[17]. Our study also proves these findings, which were very highly significant statistically.

The number and character of reticulin fibers vary considerably^[10]. In sections with abundant hemopoietic cells, there is only a slight-moderate increase in reticulin while in areas with markedly reduced hemopoietic tissue, greatly thickened, more abundant and highly intertwining bundles of reticulin fibers are recognized^[10].

We also showed that clusters of megakaryocytes are usually present and may be the only recognizable hemopoietic cells in areas of dense fibrosis. This finding

supports what is written about the close relationship between the marrow fibrosis and megakaryocytes, and the role of the megakaryocytes-derived growth factors in the pathophysiology of IMF^[13,17,20]. The marrow sinusoids are usually distended and contain hemopoietic cells. Bone trabeculae may be widened and residual fat cells may be seen in both cellular and fibrotic phase^[8].

The quantitative evaluation of bone marrow biopsies also revealed that there is a progressive reduction of hemopoietic tissue, increase in fatty tissue, fibrous tissue, OBI, TBW and blood vessels with increasing stage of IMF. This was in accordance with two earlier studies by Burchardt et al in 1982, and 1984^[13,14].

Increase number of MKCs with progression of the disease was a constant and a very highly significant finding in our study, which is consistent with previous findings reported by Jalal in 1988 who showed that collagen fibrosis was found in 86% of patients reviewed and megakaryocytes increased in numbers with tendency for clustering and abnormal morphology^[21].

References

1. Manoharan A: Myelofibrosis: Prognostic factors and treatment. *Br J Hematol*, 1988; 69: 295-8.
2. Garcia S, Miguel A, Linares M, Novarro M, and Colomina P: Idiopathic myelofibrosis terminating in erythroleukemia. *Am J Hematol*, 1989; 32: 70-1.
3. Lichtman MA: Idiopathic myelofibrosis (Agnogenic Myeloid Metaplasia). In: Lichtman MA, Beutler E, Kipps TJ, Williams WJ editors. *Williams Manual of Hematology*. 6th edition. New York: McGraw-Hill, 2003; p.p. 259-63.
4. Pearson TC, and Lewis SM: Non-Leukemic myeloproliferative disorders. In: Hoffbrand AV, Lewis SM, Tuddenham EG editors. *Postgraduate Hematology*. 4th edition. Oxford: Butter Worth-Heinemann, 1999; p.p. 520-7.
5. McCarthy DM: Fibrosis of the bone marrow: content and causes. *Br J Hematol*, 1985; 59: 1-7.
6. Kimura A, Katoh O, Hyoda H, and Kuramoto A: Transforming growth factor β regulates growth as well as collagen and fibronectin synthesis of human marrow fibroblasts. *Br J Hematol*, 1989; 72: 468-91.
7. Hasselbalch H: Pathogenesis of angiogenesis in idiopathic myelofibrosis. *Am J Hematol*, 1990; 33: 151.
8. Kaufman S, Inclea S, and Reif R: Idiopathic myelofibrosis complicated by chronic lymphatic leukemia. *Clin Lab Hematol*, 1987; 9: 81-4.
9. Hartsocck RJ, Smith EB, and Petty CS: Normal variations with aging of the amount of hemopoietic tissue in bone marrow from the anterior iliac crest. *Am J Hematol*, 1964; 43(4): 326-8.
10. Acherman RD: Bone marrow .In: Roza, J. editor. *Ackerman's surgical pathology*. 8th edition. New York.Mosby, 1995; p.p. 1797-915.
11. Thiele J, Kvasnicka HM, Diehl V, Fischer R, and Michiels JJ: Clinicopathological diagnosis and differential criteria of thrombocythemias in various myeloproliferative disorders by histopathology, Histochemistry and immunostaining from bone marrow biopsies. *Leuk Lymph*, 1999; 33: 207-18.
12. Visani G, Finelli C, Castelli U, Pett MC, Ricci P, and Vianelli N: Myelofibrosis with myeloid metaplasia: Clinical and hematological parameters predicting survival in a series of 133 patients. *Br J Hematol*, 1990; 75: 4-9.
13. Burkhardt R, Frisch B, and Bartle R: Bone marrow biopsy in hematological disorders. *J Clin Pathol*, 1982; 35: 257-84.
14. Ellis JT, Silver RT, Coleman M, and Geller SA: The bone marrow in polycythemia vera. *Seminars in Hematology*, 1975; 433-43.
15. Al-Ani RJ: Evaluation of the anemia of rheumatoid arthritis. A thesis submitted to the scientific council of pathology. 1996.
16. Lazzarino M, Morra E, Castello A, Inverardi D, Coci A, and Pagnuceo G: Myelofibrosis in chronic granulocytic leukemia: Clinicopathologic correlations and prognostic significance. *Br J Hematol*, 1986; 64: 227-40.
17. Firsch B, Lewis SM, and Burkhardt R: *Biopsy pathology of bone marrow* London: Chapman & Hall; 1985.
18. Varki A, Lottenberg R, Griffith R, and Reinhard E: The syndrome of idiopathic myelofibrosis. *Medicine*, 1983; 62(6): 353-70.
19. Wolf BC, and Neiman RS: Myelofibrosis with myeloid metaplasia: Pathophysiologic implications of the correlation between bone marrow changes and progression of splenomegaly. *Blood*, 1985; 65: 803-9.
20. Burchardt R, Bartle R, Jager K, Frisch B, Kettner G, and Mahl G: Working classification of chronic myeloproliferative disorders based on histological, hematological and clinical findings. *J Clin Pathol*, 1986; 39: 237-52.
21. Jalal ES: Fibrosis of the bone marrow pattern and significance. MSc Hematology thesis, College of Medicine Baghdad University, 1988.

22. Lee GR, Fcerster J, Lukeus J, Paraskevas F, Greer JP, and Rodgers GM: Wintrobe's Clinical Hematology New York:Williams and Wilkins, 1999.
23. Tafferri A: Proceedings of the outreach of Hematology, bone marrow transplantation. Educational Symposium, Jeddah, 1998.
24. Burkhardt R, Bartle R, and Jager K: Chronic myeloproliferative disorders. Path Res Pract, 1984; 179: 131-86.