Bone Marrow Fibrosis in Chronic myeloid leukemia (CML) and other Myeloproliferative Disorders Evaluated by Using Special Histochemical Stains for Collagen.

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Summary:

Background: It is still difficult to give a final diagnosis in chronic myeloproliferative disorders (CMPDs) because of the overlap of the common pathological and clinical features of these disorders like bone marrow fibrosis which is considered important because it affects the normal function of the bone marrow. The collagen fibers are of different types, but in the bone marrow, the two main types are: collagen I, which is the most abundant type and collagen III (reticular) which is often associated with type I.

Objectives: To study bone marrow fibrosis (BMF) in samples of bone marrow biopsies (BMB) of chronic myeloid leukemia (CML) and other chronic myeloproliferative disorders using histochemical stains to establish the grade of fibrosis and enabling a correct differentiation between chronic myeloid leukemia, essential thrombocythemia (ET), polycythemia rubra vera (PRV), and idiopathic marrow fibrosis (IMF) as subtypes of myeloproliferative disorders.

Patients and methods: This retrospective study included collection of previously preserved formalin fixed- paraffin embedded bone marrow trephine biopsies of patients with chronic myeloproliferative disorders from January 2003 through December 2008 .The relevant clinical data of patients were retrieved from the stored case sheets. Applied histochemical stains (Reticulin stain, Van Gieson stain, and trichrome stain) with Haematoxylin and Eosin (H&E) stain on sections from these specimens. These stains were used to detect the presence and the degree of pathological marrow fibrosis by the most recent grading system, the European Consensus 2005(EC2005) originally described by Thiele at 2003. Using Trichrome stain for collagen type I and reticulin stain for reticulin fibers (collagen type III) and by using a special marrow fibrosis grading system as a routine work with H&E is valuable in determining the degree of marrow fibrosis on bone marrow biopsy examination and simplifies the diagnosis.

Results: Sixty eight percent of chronic myeloproliferative disorders patients had no marrow fibrosis when diagnosed by H&E, while only 30% of chronic myeloproliferative disorders patients had no marrow fibrosis when the diagnosis was made by special stains and marrow fibrosis grading system. There is rare marrow fibrosis in essential thrombocythemia, polycythemia rubra vera, but present in chronic myeloid leukemia and almost always in marrow fibrosis. Some patients really have myelofibrosis of different grades and the histological findings by using histochemical stains are crucial to distinguish between myeloproliferative diseases

Conclusion: Patients with chronic myeloid leukemia and other chronic myeloproliferative disorders had marrow fibrosis of different grades, which is confirmed by using histochemical stains for different collagen fibers and special grading system for marrow fibrosis (EC2005) that has to be applied. It can be used routinely to avoid misdiagnosis of the primary disease or its conversion and transition to another chronic myeloproliferative disorders type, in which the clinical and laboratory features overlap, but the prognosis and therapeutic implications are significantly different.

Keywords: Chronic Myeloproliferative disorders, Myelofibrosis, Histochemical stains.

Introduction:

"Myeloproliferative disorders (MPDs)" were first defined by William Dameshek in 1951 to include chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF).1 The upcoming revised World Health Organization (WHO) classification system for hematopoietic tumors included these four classic MPDs in a broader category of "myeloproliferative neoplasm (MPNs)" that includes, in addition, other "non-classic" MPNs: chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia (CEL), hypereosinophilic syndrome (HES), systemic mastocytosis (SM), and "MPNs, unclassifiable". Both 'classic' and 'non-classic' MPNs are considered as clonal stem cell disorders. The

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molecular basis of the disease is somatic mutations, which have been identified in some but not others: *BCR-ABL* in CML, *FIP1L1-PDGFRA* and chromosomal translocations involving *PDGFRB* or *PGFR1* in molecularly-defined myeloid malignancies associated with eosinophilia. In addition, other mutations of potential pathogenetic relevance are in the process of being defined: SM-associated *KIT* mutations, Janus Kinase (JAK2V617F) in PV, ET, and PMF, Janus Kinase (JAK2) exon 12 mutations in PV, and *MPL*W515L/K in PMF and ET 2**.** The MPDs, characterized by expansion of some or all hematopoietic cell lines, are also associated with reactive proliferation of mesenchymal cells, including fibroblasts and osteoblasts. Proliferation of fibroblasts leads to increased reticulin fibers, collagen, and fibrous tissue deposition in the medullary cavity (myelofibrosis) whereas osteoblastic proliferation results in bone (osteoid) deposition in the bone marrow (osteosclerosis or osteomyelosclerosis). (3)

MPDs are syndromes characterized by a stereotypical, but often overlapping, set of pathological and clinical features. (4) There is a progressive change in the marrow histology over time. Initially, there is a proliferative phase, in which the marrow is hypercellular, all hemopoietic elements are retained, and the increased fibrous tissue consists of increased coarse reticulin in parallel bundles. Finally, an osteomyelosclerotic stage is reached, in which there is replacement with highly vascular fibrous tissue containing new bone and markedly dysplastic megakaryocytes. 5 (table 1)

Table (1): Comparative features and laboratory findings of classical MPD 6

Feature	CML	ET	PV	CIMF
Overproduction of 1 or more blood cell lines with dominance of a transformed clone	+	+	+	+
Increased fibrosis in bone marrow	≤40%	-	+/-	+
Increased cellularity in bone marrow	+	+/-	+	+/-
Chromosomal abnormalities	100%	~5%	~15%	~35%
Thrombotic and/or hemorrhagic complications	-	+	+	+
Extramedullary hematopoiesis (EMH) by liver and/or spleen	+	+/-	+	+
Transformation to AML	~70%	<5%	~10%	~ 18%
Overlapping clinical features	+	+	+	+

Pathogenesis of marrow fibrosis: The increased content of marrow collagen types I and III results from release of fibroblast growth factors, which include PDGF (platelet derived growth factor), epidermal growth factor, endothelial cell growth factor. 7-8 other factors, such as tumor necrosis factor alpha, IL-1, and IL-6, which can be released from marrow cells, also can stimulate fibroblasts. Platelet factor 4, also derived from megakaryocytes, inhibits collagenase and could contribute to collagen accumulation, although studies showing a poor correlation between plasma platelet factor 4 concentrations and marrow fibrosis have dampened enthusiasm for the role of this factor. 9-10-11 Substance P, a peptide that acts as a neurotransmitter and a modulator of immune and hematopoietic functions, is increased in the fibrotic marrow and localizes with fibronectin. It is angiogenic and is a fibroblast mitogen. 12 Its precise role in the complex interactions among fibroblasts, cytokines, and matrix protein deposition is not clear. The high urinary excretion of platelet-derived calmodulin, a putative fibroblast growth factor, in patients with myelofibrosis has added this compound to the array of factors that may contribute to the fibroplasia. (11) Plasma level of matrix metalloprotein III is decreased and the level of tissue inhibitor of metalloproteinase is increased in patients with idiopathic myelofibrosis. (13)

Patients and methods:

Patients: The selection of patients in this study was made by taking all bone marrow biopsies (BMB) of MPD patients performed in the period between the first of January 2003 and the first of January 2008 and were collected at 4 months duration in Medical City Teaching Hospital (MCTH) laboratories by re-evaluation for any evidence of marrow fibrosis in all diagnosed cases of myeloproliferative disorders. The total number of positive bone marrow biopsy for myeloproliferative neoplasia was [154 cases]. Twenty two cases were excluded (Deficient essential clinical data in the medical records (17 cases), repeated BMB specimen for follow up of already diagnosed patients (2 cases), Inadequate BMB specimens including autolysed specimens, fragmented specimens, very poorly processed specimens and very tiny specimens (3cases). This exclusion process left us with (132) cases included in this study.

Clinical & hematological studies: The clinical records of all the patients included in this study were carefully re-evaluated. The peripheral blood & bone marrow aspirates smears were re-evaluated for all patients included in this study over this period. The full blood counts were all done at Hematology Lab. of Medical City Teaching Laboratories (MCTL) by routine manual methods, blood & marrow smears were stained using Leishman's stain. Reticulin stain (RS), Masson's trichrome stain, and Van Gieson stain, were done for all of the included BMB in this study, using the original stored BMB paraffin blocks. The processing of the BMBs was done in the histopathology Lab. of the MCTL, while staining & mounting of the BMB slides were done in the

MTCL and the department of pathology – College of Medicine – University of Baghdad.

Grading, Sectioning and Staining : For each BMB paraffin block, four sections were done & each one was cut at about 5 µm thickness, and different types of stains were done (Table2), and European Consensus grading system (EC)2005 grading system was applied (Table3).

Table 2: Types of stains

No.	Stain	Purpose
1	Haematoxylin & Eosin stain (H & E)	For demonstration of cytological details marrow pattern, pathological changes as fibrosis.
EC2005 grading system		
1	Gomoris stain (Reticulin stain RS)	For demonstration of the presence of reticulin fibers, collagen type III, black color
2	Masson's Trichrome stain (Bouin's stain)	For demonstration of the presence of collagen fibers type I, blue color

Table 3: EC 2005 for bone marrow fibrosis using Reticulin special stains for Reticulin (type III collagen) and Trichrome stain for Collagen type I fibers

Grading	Description
MF-0	Scattered linear Reticulin with no intersections(cross-over) corresponding to normal bone marrow
MF-I	Loose network of Reticulin with many intersections, especially in peri-vascular areas
MF-II	Diffuse and dense increase in Reticulin with extensive intersections, occasionally with only focal bundles of collagen and/or focal osteosclerosis
MF-III	Diffuse and dense increase in Reticulin with extensive intersections with coarse bundles of collagen ,often associated with significant osteosclerosis

Results:

In this study, we studied the relationship between MPD and the presence of pathological marrow fibrosis.

In CML, seventy one out of 99 patients with CML had pathological marrow fibrosis; all patients (100%) with accelerated phase of CML had pathological marrow fibrosis with a median grade of MF-II, while the other patients in chronic and blastic phase had marrow fibrosis with a median grade of MF-I, meanwhile (84.4%) and (69%) of patients had pathological marrow fibrosis in chronic phase and blastic phase respectively.

Twelve patients (92.3 %) out of 13 with primary myelofibrosis have a median grade of MF-I. Regarding the ET, six patients (55%) with ET had no marrow fibrosis and 3 patients (25%) had grad MF-I, while 2 patients (20%) had MF-II. Three out of 5 PV patients (60%) had MF-0, While 2 patients (40%) had MF-I or MF-II. ET and PRV have the lowest rate to have marrow fibrosis with a median grade of MF-0. The association between different types of myeloproliferative disorders and the pathological marrow fibrosis grades failed to reach the level of statistical significance possibly because of small sample number, while there is an association between the median of marrow fibrosis grading and the types of myeloproliferative disorders with a P value of 0.04 (Table 4).

Table 4: the association between different types of MPD and the presence of pathological marrow fibrosis as well as median for marrow fibrosis grading

	Diagnosis	Total 132 patients	Pathological fibrosis (93 patients)		Median for BM Fibrosis grading
		N	N	%	
1	Essential thrombocythaemia	11	5	45.5	MF-0
2	Polycythaemia Vera	5	2	40	MF-0
3	Primary myelofibrosis	13	12	92.3	MF-I
4	Unclassified MPD	4	3	75	MF-I
5	Chronic phase CML	84	58	69	MF-I
6	Blastic phase CML	13	11	84.6	MF-I
7	Accelerated phase CML	2	2	100	MF-II
	P value		0.1[NS]		0.04

P value <0.05 is significant

The difference in the diagnosis of marrow fibrosis between ordinary Hematoxylin /Eosin stain (H&E) and European Consensus (EC) grading system. In this study, the H&E stain revealed that ninety patients (68.8%) with MPD have no pathological marrow fibrosis, while EC2005 grading system revealed that thirty nine patients(29.5%) have no pathological marrow fibrosis. Twelve patients (9%) were diagnosed to have severe marrow fibrosis by H&E stain while EC2005 grading system revealed only five patients (3.8%) to have grade MF-

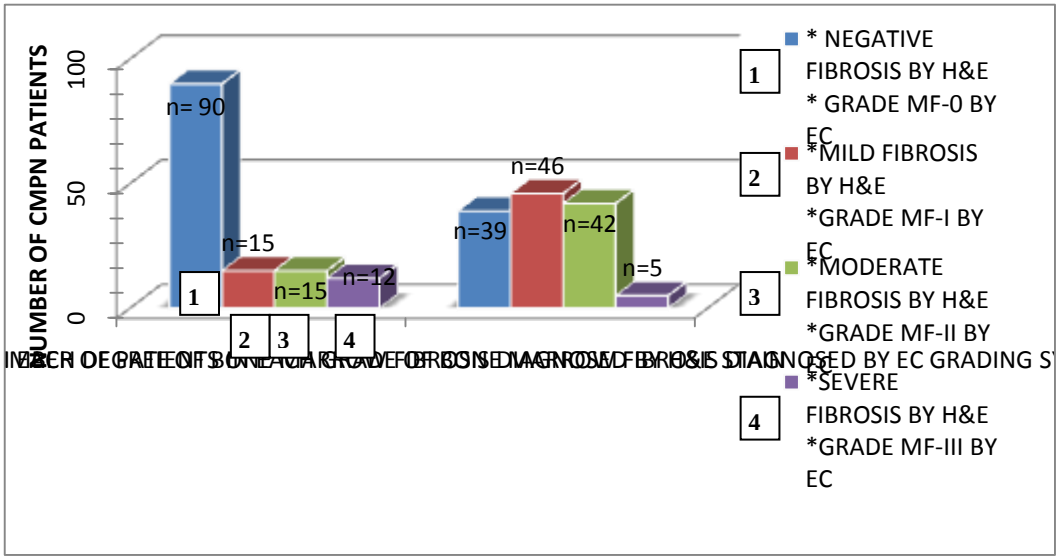

Figure 1 Difference between EC grading system and H&E stain in detection of marrow fibrosis

Discussion:

The use of reticulin stain and trichrome stain was effective when compared with the use of H&E stain alone as a laboratory diagnostic method for detection and grading of marrow fibrosis in MPD patients. In this study, we noticed that 68.8% of our previously diagnosed patients had no marrow fibrosis by using only H&E stain. The H&E stain identifies only MPD patients without determining the exact degree of marrow fibrosis, which was important for the definite diagnosis and evaluation of effectiveness of therapy and transformations in MPD. In previous study, marrow fibrosis resulted in a significant shortening of survival times of patients, independent of the type of therapy applied including allografting. 14. Also, in this study, we noticed that the H&E detects a higher percentage of severe marrow fibrosis (9%) than the MF-III in the EC grading system (3%). This might be due to the presence of fibroblastic reaction by H&E without production of collagen fibers. Collagenase-1 [matrix metalloproteinase (MMP-1) or fibroblast collagenase], collagenase-2 (MMP-8 or neutrophil collagenase, and collagenase-3 (MMP-13) constitute a small group of proteases within the MMP family that can cleave fibrillar interstitial collagens at neutral pH.15-16 Collagenase-3 was identified as a novel collagenase in human breast carcinoma and was found to be expressed in certain normal cells, including rat and human osteoblasts and chondrocytes and serves as tissue remodeling . Basic fibroblast growth factor (bFGF) stimulates collagenase-3 synthesis in fetal rat osteoblast-enriched (Ob) cells.17, Data on serum markers of collagen metabolism and expression of members of the urokinase-type plasminogen activator system (uPA) suggested an impact on remodeling processes during myelofibrosis 18

Conclusions:

Bone marrow fibrosis might be a hidden condition in MPD patients which may be negative due to the use of H&E stain only as an initial diagnosis of the disease, the use of a grading system in marrow fibrosis is simple to identify the reticulin and collagen fibrosis which is essential in giving the more precise status of marrow fibrous contents (pathological or physiological) in MPD patients and to distinguish between myeloproliferative diseases or the transition to another hematological condition and some essential thrombocythemia patients who were diagnosed by H&E only, actually were in early stage chronic idiopathic marrow fibrosis which is common in CML, rare in PRV and ET.

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