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Research

Reactive Changes in Bone Marrow Biopsies: A Clinicopathological Study

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Abstract

Background

Many diagnostic challenges arise due to insufficient experience with reactive bone marrow changes which can be sometimes confused with neoplastic disorders.

Aim

To identify various reactive changes affecting bone marrow cells and stroma in response to different neoplastic and non neoplastic disorders in bone marrow biopsies

Methods

We carried out retrospective analysis of bone marrow reactive changes in 205 trephine biopsies obtained at Oncology centre, Mansoura University, Egypt from 2010 to 2016. Four micrometer thick sections were stained with H&E. Reticulin stain was used for grading of myolefibrosis. Immunohistochemical staining of CD20 and CD3 were used to confirm reactive lymphoid proliferation. CD138 and CD56 was used to assess the percentage and the neoplastic nature of plasma cells.

Results

The patient's clinical disorders were grouped into neoplastic disorders that included 113 cases (55.1%) and non neoplastic disorders included 92 cases (44.9%). Cytopenias were the most common findings in peripheral blood picture. Reactive lymphoid proliferations (confirmed by immunohistochemical staining for CD20 and CD3) were detected in 23.9 % of cases , reactive plasmacytosis (confirmed by immunohistochemical staining for CD138) in 11.7 %, erythroid hyperplasia in 22.9 % of cases, megakaryocytes hyperplasia in 11.7 %, myeloid hyperplasia in 7.8%, marrow hypoplasia and aplasia in 26.3 % and 7.8% respectively, secondary dysplasia in 22 % and myelofibrosis in 25.9%. Bone marrow aspirate showed positive correlation with biopsy in detection of reactive changes.

Conclusion

We concluded that many reactive changes occur in bone marrow in response to different neoplastic and non neoplastic diseases.

Keywords: Bone Marrow; Reactive; Parenchyma, Stroma

Introduction

Bone marrow (BM) examination is an integral component of the assessment of different hematological and non-hematological disorders [1]. Many conditions such malignancies, infections and immune disease modify the cellular and interstitial component of marrow which can be studied in bone marrow aspiration and biopsy [2]. Knowledge of the normal composition of BM and its normal variations is required for accurate morphological examination of bone marrow biopsies (BMBs), however diagnostic challenges may arise due to insufficient experience with reactive BM changes which can be sometimes confused with neoplastic disorders [3].

BM reactive changes can affect quantity or quality of one or more hematopoietic cell lines, or affect the BM stroma [3]. Distinction between reactive marrow changes and neoplastic infiltrates needs an integrated approach combining clinical and laboratory data with BMB findings together with the results of other ancillary tests [4]. Reactive changes can be in the form of hyperplasia involving one or more cell lines mostly in response to peripheral consumption or intrinsic marrow insult or it can take the form of hypoplasia or even aplasia. Secondary dysplastic changes can also occur as an effect of therapy or neoplasms involving the

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marrow [5] and their differentiation from myelodysplastic syndromes can be problematic in some cases [4]. On the other hand, bone marrow stroma may respond to different diseases as in cases of secondary myelofibrosis (MF) [6].

Hyperplasia can involve lymphoid or myeloid series. Reactive lymphoid proliferations may be either an interstitial increase in lymphocytes or take the form of lymphoid aggregates [7]. Benign lymphoid aggregates (BLAs) although seen only in a minority of BM specimens, they are diagnostically significant as they have to be distinguished from non-Hodgkin lymphoma (NHL) infiltrates, particularly B-cell lymphomas [8]. Moreover reactive plasmacytosis that can be seen in various conditions have to be differentiated from plasma cell dyscrasis [9].

Furthermore, myeloid reactive proliferations including erythroid, granulocytes, monocytes and megakaryocytes hyperplasias are also seen as a response to hematopoietic and non hematopoietic disorders [10] and their differentiation from myeloproliferative neoplasms should be considered [11].

So, the aim of our study was to identify various reactive changes affecting bone marrow cells and stroma in response to different neoplastic and non neoplastic disorders in bone marrow biopsies.

Table 1: Clinical history of all studied cases.

Clinical history		No	%
Non neoplastic (n=92)	Chronic disease [1]	51	55.4%
	Chronic infections[2]	22	23.9%
	Non neoplastic hematological disorders[3]	19	20.7%
Neoplastic (n=113)	Lymphoma[4]	93	82.3%
	Hematological neoplasms other than lymphoma[5]	5	4.4%
	Non hematological neoplasms[6]	15	13.3%

1: Chronic diseases included: chronic liver diseases, chronic renal diseases, immune disorders,

2: Chronic infections included: Hepatitis B, C virus, bilharziasis,

3: Non neoplastic hematological disorders: Castelman disease, ITP, cytopenias and polycythemia, thrombocytosis,

4: Lymphoma cases may have another associated chronic infections or chronic diseases

5: Hematological neoplasms other than lymphoma: Multiple myeloma, acute myeloid leukemia, myelodysplastic syndrome and Langerhans cell histiocytosis,

6: Non hematological neoplasms: cancer breast, cervix, larynx, thyroid, liver and neuroblastoma.

Material and Methods

We carried out retrospective analysis of bone marrow reactive changes in trephine biopsies obtained for various indications at Oncology centre, Mansoura University, Egypt from January 2010 to January 2016. Patient's age, sex, clinical presentation were recorded. Results of peripheral blood picture (PBP) and bone marrow aspirates (BMA) were reviewed. Total number of 205 bone marrow biopsies were evaluated. The bone marrow biopsies obtained by Jamshidi needle (8G x 10cm) were fixed in 10% formalin solution, decalcified by formic acid 10% and paraffin embedded. Four micrometer thick sections were stained with hematoxylin-eosin. Reticulin stain was used for grading of MF. Immunohistochemical staining of CD20 and CD3 were used to confirm reactive lymphoid proliferation. CD138 and CD56 to assess the percentage and the neoplastic nature of plasma cells.

Results

The study included 116 males and 89 females with age ranged from 2 to 79 years with mean age 45.68±16.93 SD. The patient's clinical disorders were grouped under two main headings: neoplastic disorders that included 113 cases (55.1%) and non neoplastic disorders included 92 cases (44.9%) and each category included different disease entities which were illustrated at table (1). Among neoplastic disorders, lymphomas were the major group included 93 cases (82.3%) of total neoplastic cases.

As regard peripheral blood picture; cytopenias were the most common findings. All cases of lymphocytosis had history of lymphoma. The peripheral blood picture was statistically significant between neoplastic and non neoplastic cases (P value<0.001) [Table 2].

Table 2: Peripheral blood picture in neoplastic and non neoplastic conditions.

CBC findings	Non neoplastic (n=92)		Neoplastic (n=113)		P
	No	%	No	%	
Normal	4	4.3%	27	23.9%	<0.001*
Cytopenias	71	77.2%	61	54.0%	
Lymphocytosis	0	0.0%	18	15.9%	
Leucocytosis	4	4.3%	6	5.3%	
Thrombocytosis	3	3.3%	1	0.9%	
Elevated hemoglobin	7	7.6%	0	0.0%	
Eosinophilia	3	3.3%	0	0.0%	

P:Probability *:significance <0.05 Test used:Pearson's chi-square

As regard bone marrow aspirate, only 157 cases (76.6%) showed adequate aspirates while 48 cases (23.4%) showed diluted aspirates or dry taps. Neoplastic and non neoplastic cases varied significantly in their cellularity and other BMA findings (P value<0.001, 0.01 respectively) [Table3].

Table 3: Bone marrow aspirate findings in neoplastic and non neoplastic conditions.

Bone marrow aspirate		Non –neoplastic (n=92)		Neoplastic (n=113)		P
		No	%	No	%	
Cellularity	Normocellular	20	21.7%	48	42.5%	<0.001*
	Hypocellular	18	19.6%	5	4.4%	
	Hypercellular	36	39.1%	30	26.5%	
	Dry tap or diluted	18	19.6%	30	26.5%	
Findings	Normal	31	33.7%	44	38.9%	0.01*
	Erythroid H**	23	25.0%	18	15.9%	
	Megakaryocytic H**	6	6.5%	2	1.8%	
	Increased lymphocytes	1	1.1%	9	8.0%	
	Plasmacytosis	2	2.2%	2	1.8%	
	Eosinophilia	1	1.1%	2	1.8%	
	Hypoplasia	10	10.9%	3	2.7%	
	Malignant cells	0	0.0%	3	2.7%	
	Dry tap	18	19.6%	30	26.5%	

P:Probability *:significance <0.05 Test used:Pearson's chi-square **Hyperplasia

Various reactive paremchymal changes were detected in BMB. Reactive lymphoid proliferation were found in the form of 1-reactive lymphoid aggregates (mixed B and T lymphocytes confirmed by immunohistochemical staining for CD20 and CD3) in 8 cases of the non-neoplastic disorders and 17 cases of the neoplastic disorders (figures 1-4). 2-reactive scattered T lymphocytosis (confirmed by immunohistochemical staining for CD3) in exclusively 17 neoplastic lymphoma cases. 3-reactive interstitial mixed lymphocytosis (confirmed by immunohistochemical staining for CD20 and CD3) in 4 cases of the non-neoplastic disorders and 3 cases of the neoplastic disorders [Table 4].

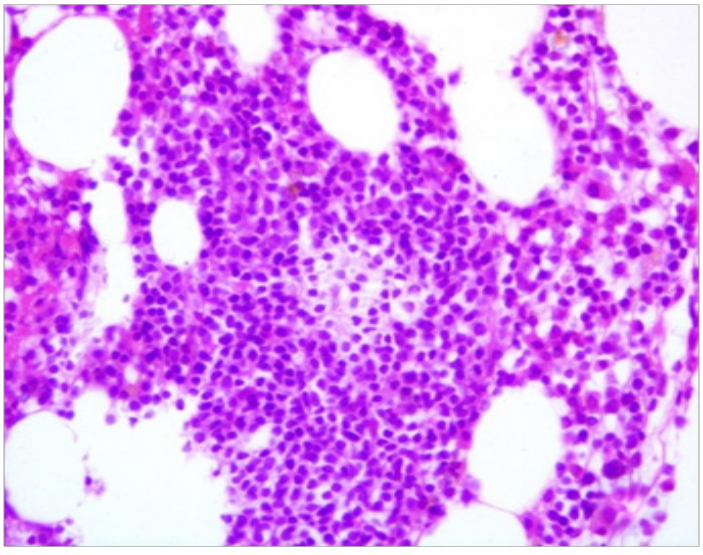

Figure 1: Reactive lymphoid aggregate with germinal center in cirrhotic patient(H&E x400)

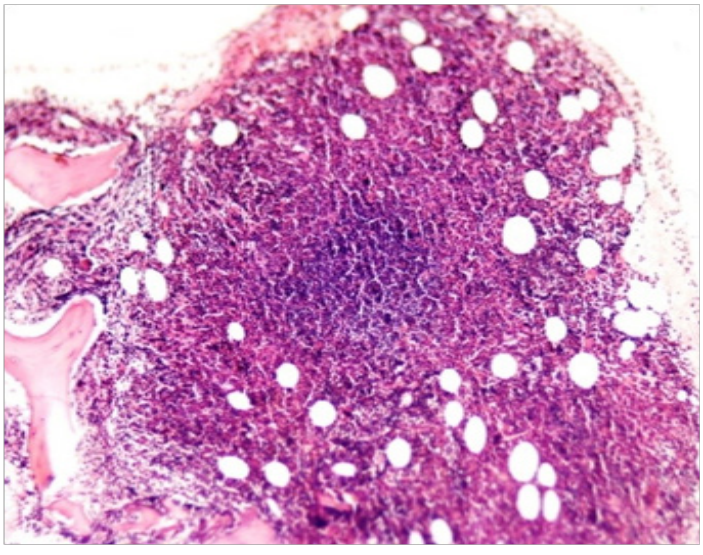

Figure 2: Reactive lymphoid aggregate in a case of MDS, (H & E x 100).

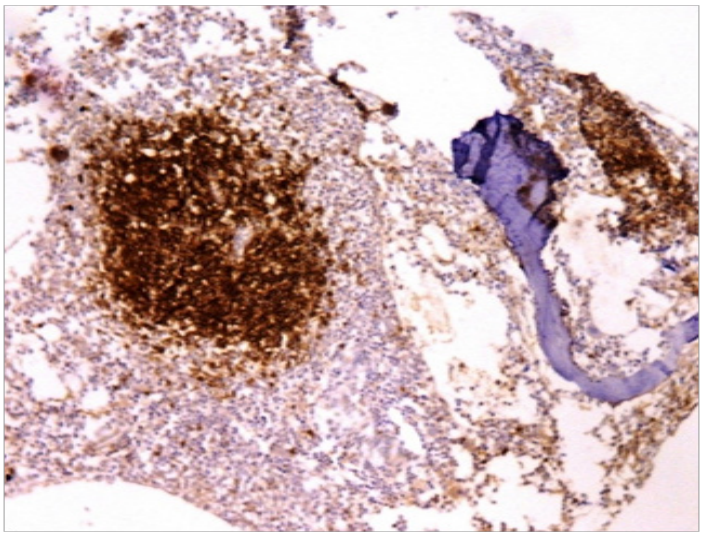

Figure 3: Reactive lymphoid aggregate with mixed B and T lymphocytes pattern (CD20 x200).

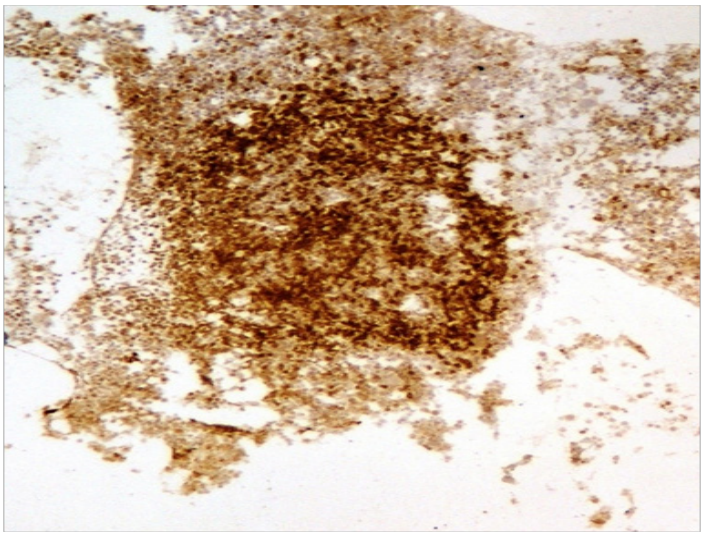

Figure 4: Reactive lymphoid aggregate with mixed B and T lymphocytes pattern (CD3 x200).

Table 4: Reactive parenchymal and stromal bone marrow changes in response to neoplastic and non neoplastic conditions

Reactive parenchymal bone marrow changes	Non – neoplastic		Neoplastic		P
	No	%	No	%	
Reactive lymphoid proliferation(n=49)					
Reactive lymphoid aggregates(mixed B &T)	8	66.7%	17	45.9%	0.006*
-Reactive scattered T lymphocytosis	0	0.0%	17	45.9%	
-Reactive scattered B or mixed lymphocytosis	4	33.3%	3	8.1%	
Plasma cell %(n=205)					
<5%	78	84.8%	103	91.2%	0.3
5-10%	4	4.3%	2	1.8%	
>10%	10	10.9%	8	7.1%	
Reactive erythroid series(n=205)					
Normal	24	26.1%	62	54.9%	<0.001*
Hypoplasia	17	18.5%	24	21.2%	
Hyperplasia	31	33.7%	16	14.2%	
Dyserythropoiesis	20	21,7%	11	9.7%	
Granulocytic series (n=205)					
Normal	51	55.4%	77	68.1%	0.14
Hypoplasia	30	32.6%	29	25.7%	
Hyperplasia	9	9.8%	7	6.2%	
Dysplasia	2	2.2%	0	0.0%	
Megakaryocytes series(n=205)					
Normal	23	25%	59	52.2%	<0.001*
Hypoplasia	27	29.3%	29	25.7%	
Hyperplasia	12	13%	12	10.6%	
Dysplasia	30	32.6%	13	11.5%	
Fibrosis					
G0-2	70	85.9%	73	64.6%	0.001*
G3	8	8.7%	15	13.3%	
G4	5	5.4%	25	22.1%	

Reactive plasmacytosis was detected in twenty four cases (11.7%) with percentage ranged from (5 - >10%) [Table 4]. Histopathologically; these cases showed mature plasma cells that attained perivascular locations (figure 5) or occurred in small clusters. CD138 highlighted the plasma cells and determined their percentage (figure 6). All cases were negative for CD56.

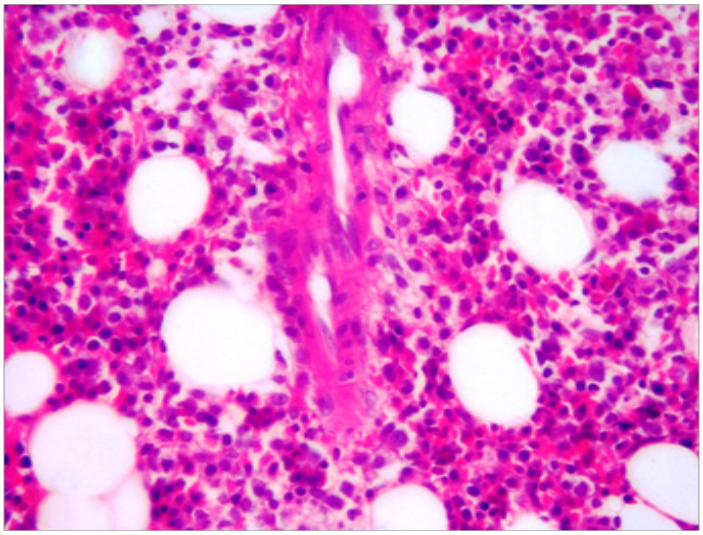

Figure 5: Perivascular arrangement of reactive plasma cells in cirrhotic patient, (H & E x400).

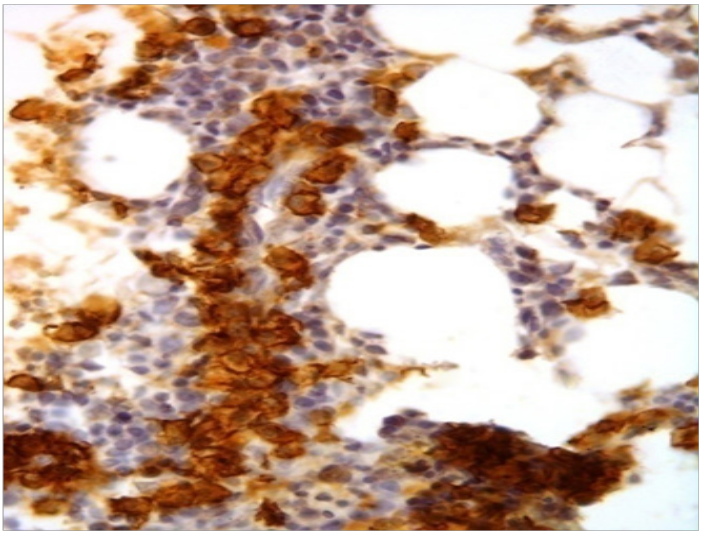

Figure 6: CD138 highlighted the perivascular plasmacytosis (CD138 x400).

Erythroid series showed hyperplasia, hypoplasia or dysplasia in response to various neoplastic and neoplastic disorders [Table 4]. Erythroid hyperplasia was detected in 47 cases (22.9%) (Figure 7).

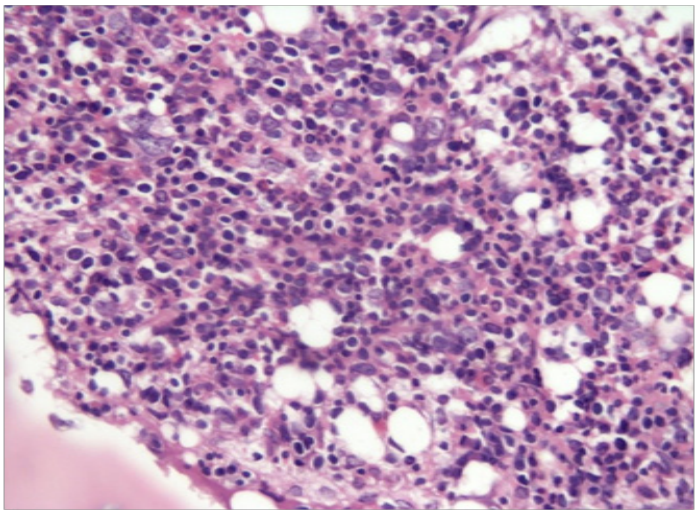

Figure 7: Erythroid hyperplasia with dyserythropoiesis in Autoimune hemolytic anemia, (H & E x400).

Granulocytic hyperplasia was present in 16 cases (7.8%), 12 out of them showed dominant eosinophilic series. They included 2 cases of Langerhans cell histiocytosis (figure 8), 3 cases of Hodgkin lymphoma without BM involvement, 2 cases of non Hodgkin lymphoma, 2 cases of HCV, 2 cases with fever of unknown origin and one case with bilharzial infection. Two cases of histiocytic proliferations were also detected; one in the form of epitheloid granuloma detected in a case of Hodgkin lymphoma (figure 9) and the other was hemophagocytic syndrome in a case of acute myeloid leukemia.

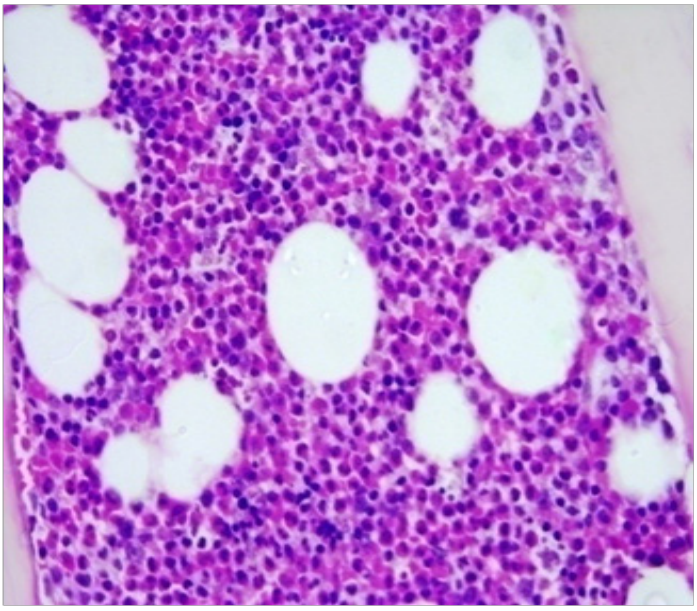

Figure 8: Reactive eosinophilic hyperplasia in Langerhans cell histiocytosis without BM involvement, (H & E x 400).

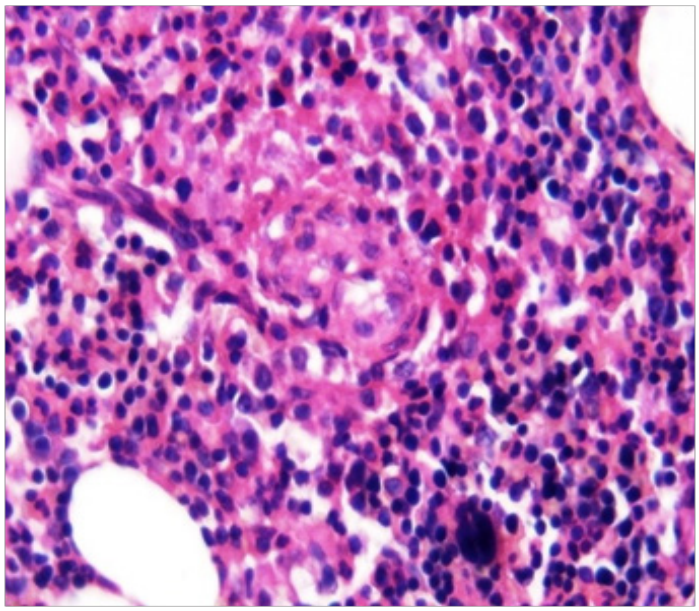

Figure 9: Epitheloid granuloma in a patient with Hodgkin lymphoma, (H & E x 400).

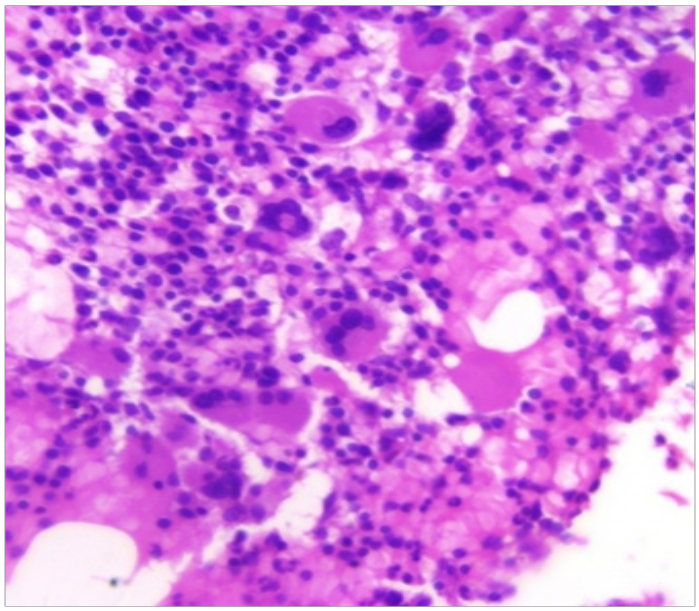

Figure 10: Reactive megakaryocytic hyperplasia in patient with peripheral platelets destruction, (H & E x400).

Megakaryocytes hyperplasia was detected in twenty four cases (11.7%) [Table 4] (figure 10), twenty of them were thrombocytopenic and four demonstrated thrombocytosis on blood picture.

Aplastic anemia (AA) was detected in sixteen cases representing (7.8%) of total studied cases. They showed hypocellular BM on aspirate in 62.5% of cases, 25% were dry tap and only 12.5% showed cellular marrow. Bone marrow biopsies of these cases showed reduction in all series (figure 11) except for three cases which demonstrated erythroid hyperplasia and one case showed reactive plasmacytosis. Secondary MF was detected in 53 cases (25.9%) [Table 4], (figure 12).

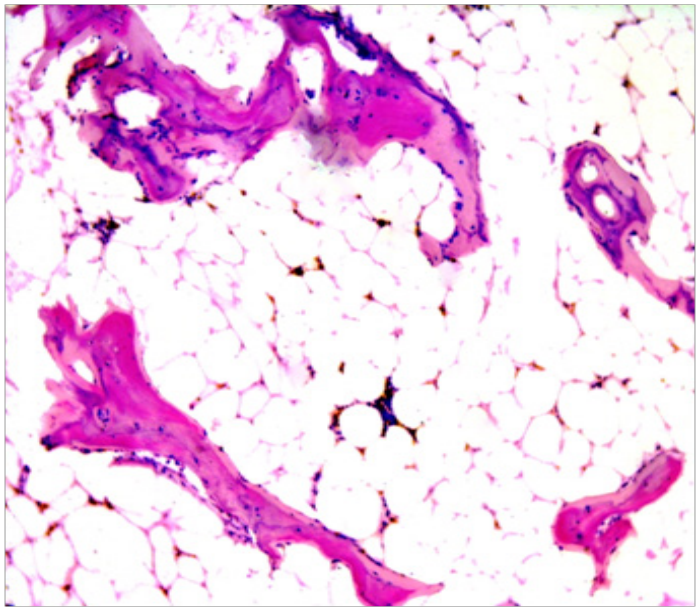

Figure 11: Bone marrow failure, in renal transplant patient, (H&E x100).

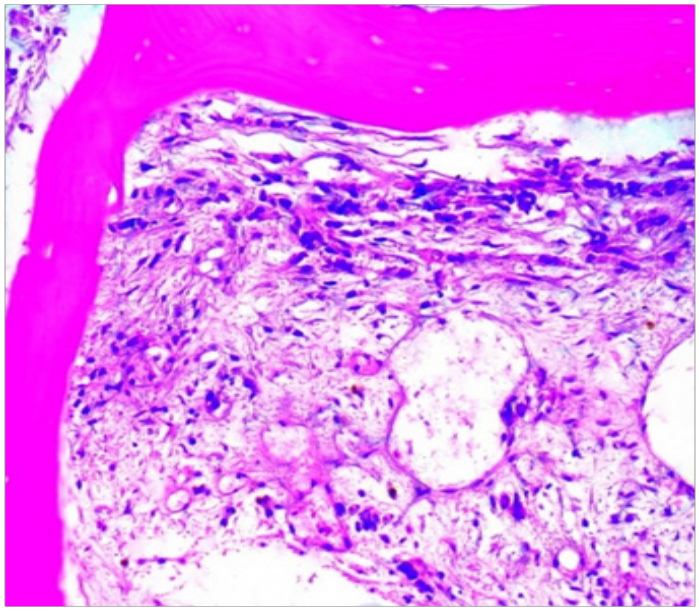

Figure 12: Myelofibrosis in case of metastasis of unknown origin, (H&E x400).

Reactive changes affecting lymphoid (P value=0.006) ,erythroid (P value<0.001), megakaryocytes series (P value<0.001) and MF (P =0.001) showed statistical significant difference between neoplastic and non neoplastic disorders [Table 4].

BMA in the present study showed positive correlation with biopsy in detection of reactive changes in (39.5%) of cases having adequate BMA. Cases with erythroid hyperplasia showed the highest positive correlation (87.2%) as shown in table 5.

Table 5: Comparative evaluation of bone marrow aspiration and bone marrow biopsy in diagnosis

	BMA	BMB	Diagnostic accuracy of BMA
Over all positive correlation	62	157	39.5%
Reactive lymphoid proliferations	4	49	8.2%
Reactive plasmacytosis	4	24	16.7%
Reactive erythroid hyperplasia	41	47	87.2%
Reactive megakaryocytic hyperplasia	8	24	33.3%
Reactive granulocytic hyperplasia	3	16	18.8%
Hypoplasia	13	54	24.1%
Myelofibrosis	-	53	-
Lymphomatous deposits	6	27	22.2%

N.B: Cases with dry tap or diluted aspirates were excluded, one case may have more than one change

Discussion

This study was conducted on 205 BM trephine biopsies showing various reactive changes involving BM parenchyma and stroma, in response to different diseases. Our study showed that reactive changes detected in BM were not disease specific and this was compatible with Diebold et al, [12] study on BM changes in infections and systemic diseases who stated that morphologically similar bone marrow lesions can arise from different pathological agents and one disease can cause several different marrow lesions.

In this study, the clinical disorders, that contributed to the reactive bone marrow changes, were grouped into neoplastic and non neoplastic disorders. Lymphoma was the most commonly detected neoplastic disorder (82.3% of the neoplastic disorders). This could be explained by the fact that most BMBs were done for assessment of lymphoma patients. This was in agreement with Picken et al, [13] who stated that the pathological assessment of the BM plays a central role for diagnosis, prognosis, monitoring and management of malignant lymphomas and this is considered an important indication for BMB [14].

In the current study, reactive lymphoid hyperplasia account for 23.9% of total studied cases. Lymphoid cell hyperplasia has been described in numerous different diseases but with a highly variable frequency from one publication to another. Brunning and MacKenna, [15] in a survey of the literature reported a frequency of (18-47%) in trephine BMB. Reactive lymphoid proliferations in our study were found in different neoplastic and non neoplastic clinical situations. They present either in the form of aggregates or scattered interstitial lymphocytes. This was consistent with Jaffe et al., [7] that stated that increased marrow lymphocytosis may be either an interstitial increase in lymphocytes or occur in the form of lymphoid aggregates. Reactive scattered T lymphocytosis was found in all cases of lymphoma. This is similar to Kremer et al., [16] who found a high number of reactive T cells in neoplastic infiltrates of BM in B-cell lyphomas. Also, Schirrmacher, [17] reported that increased reactive memory T cells in BM was observed in patients with hematological neoplasms even without BM involvement due to activation by blood borne tumor antigens.

Regarding cases of reactive plasmacytosis, none showed cellular atypia and all were CD56 negative. This was in agreement with Anagnostou, [18] who stated that atypia is more often seen in neoplastic plasma cells but not in reactive ones which are polyclonal and typically CD56 negative however clonality was not assesses in any of our cases due to financial limitations.

In this study, reactive changes involving myeloid lineage occurred in response to neoplastic and non-neoplastic disorders were in the form of hyperplasia, hypoplasia or dysplasia. This was in match with Rosi and Ackermann, [10] who stated that hyperplasia of one or more myeloid cell lines (erythroid, granulocytic, megakaryocytic) may be found in several non-neoplastic (such as megaloblastic and sideroblastic anemias) and neoplastic hematopoietic disorders (reactive megakaryocytosis seen in bone marrows infiltrated by lymphoma). Exact frequencies of these changes are lacking in literatures.

Moreover, secondary dysplasia involving myeloid lineage have been observed in neoplastic and neoplastic disorders. This was in agreement with Amos et al, [19] who stated that myelodysplasia can occur secondary to hepatic disorders, autoimmune diseases, multi organ failure and lymphoproliferative disorders.

In our study, AA was detected in 7.8% of total cases. They showed hypocellular BM on aspirate in 62.5% of cases. Our results were matched with the study of Khan et al., [20] who found that 70% of cases of AA showed hypocellular marrow on aspirate smear. BMB of these cases in the current study showed reduction in all series except for three cases demonstrated erythroid hyperplasia and one case show reactive plasmacytosis. This was in agreement with Milosevic et al, [21] who

stated that BM in AA is markedly hypocellular with residual few reactive inflammatory infiltrate including plasma cells.

On the other hand, one of the common stromal reactive changes in BM was MF [6]. Increased BM stromal fibers occur as a response to various benign and malignant disorders [22] and this was in agreement with our results in which grade 3 and 4 MF were detected in 35.4% of neoplastic cases and 14.1% of non neoplastic cases.

BMA in present study showed positive correlation with bone marrow biopsy in detection of reactive changes in 39.5% of cases. This was in contrast with Mahajan et al, [1] who showed that BMA diagnosed 97.5% of the cases of reactive BM picture. Furthermore, khan et al, [20] observed 73.8% positive correlation between BMA and trephine biopsy and also 78% positive correlation have been reported by Chandra et al., [23]. This wide variations could be attributed to that most of our cases displayed more than one reactive change in which some of them could not be detected in BMA while the compared studies dealt with reactive changes as one finding without specify the type of that reactive change. In addition, this study showed highest positive correlation in the diagnosis of reactive erythroid hyperplasia (87.2%) that was in agreement with Khan et al study.

Conclusion

We concluded that many reactive changes occur in bone marrow in response to different diseases and the reactive nature in these cases cannot be confirmed except through an integrated approach combining clinical data with results of peripheral blood and bone marrow aspirate and other immunohistochemical and ancillary studies.

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