Original Article

Role of Bone Marrow Clot Biopsy in Workup of Haematological and Non-Haematological Diseases

Diagnosis of Haematological and non Haematological Disorders from Bone Marrow

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ABSTRACT

Objective: To study the role of bone marrow clot biopsy in the diagnosis of haematological and non haematological disorders.

Study Design: Descriptive cross sectional

Place and Duration of Study: This study was conducted at the Department of Pathology, Ayub Medical College from January 2018 to December 2019 over two years.

Materials and Methods: 335 patients of both sex and age > two years were enrolled by non-probability convenience sampling after an informed written consent. Children <two years old were excluded. Venous blood sample taken for complete blood counts and blood film, bone marrow obtained from posterior iliac spine, smears made from the marrow aspirate and venous blood immediately and fixed in alcohol after drying. The remaining part of bone marrow sample was allowed to clot and fixed in 10 percent buffered formol saline and processed further like soft tissue biopsy. Trephine biopsy obtained from the same site, fixed in 10 percent formol saline, before processing further. Slides prepared from blood were stained by May Grunewald Giemsa (MGG). Slides of bone marrow trephine biopsy and clot biopsy were stained by haematoxylin & eosin stain after processing. These slides were examined independently by an experienced pathologist taking care of biasness and double blinding. Results of bone marrow clot biopsy were compared with trephine biopsy.

Results: Total of 335 patients (176 males and 159 females, Male to female ratio 1:1.1). Trephine biopsy made conclusive diagnosis in 326 and marrow clot biopsy in 121 patients.

Conclusion: There is statistically no significant difference in diagnostic role of trephine and clot biopsy.

Key Words: Bone marrow, trephine biopsy, marrow clot biopsy.

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INTRODUCTION

Bone marrow examination plays a pivotal role in the diagnosis of a number of haematological conditions like anaemia, leukaemia, thrombocytopenia, myeloproliferative neoplasms as well as non haematological conditions like metastatic cancers, tuberculosis, pyrexia of unknown origin and osteopetrosis[1-6]. Patients coming to physicians, who are advised bone marrow examination, may benefit more if they are referred first to a haematologist to evaluate the need for this test. It may not be beneficial many a times[7].

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Investigational procedures including bone marrow may be made more useful if primary focus is on treatable underlying pathology[8]. The knowledge of normal histology of bone marrow, reactive changes, age related changes and use of newer techniques in addition to the basic clinical and haematological information may be more fruitful than simply focusing upon bone marrow findings only[9,10]. Marrow in young children with visceral leishmaniasis may show dyserythropoiesis and prominent plasma cells, which may be confused with pathology of haemopoiesis if care is not observed[11]. Bone marrow examination has two parts, a fluid part known as bone marrow aspirate and a solid part which is called trephine biopsy. It comprises examination of marrow aspirate (fluid in nature) and trephine biopsy (solid cylinder of marrow). Samples for both the parts are obtained in one sitting. Bone marrow aspirate may not be enough to reach a conclusive diagnosis in conditions like multiple myeloma, lymphoma, metastatic bone disease and aplastic anaemia. A good-length trephine biopsy is necessarily taken if any of these conditions is suspected. Studies have revealed improved diagnostic yield if both the aspirate and trephine biopsy are taken simultaneously[12]. The

marrow aspirate is smeared on slides, fixed and examined by the haematologist, while trephine biopsy is processed like a histopathology sample is also examined by histopathologist. The aspirated material left behind after making adequate number of slides is usually discarded. For the last few years, it has been observed that if is allowed to clot and processed like a soft tissue biopsy specimen for histopathology (also called bone marrow clot biopsy), it may provide valuable information regarding final diagnosis[13-16]. It has also been learnt in the past years that examination of bone marrow clot biopsy, may also obviate the need of performing trephine biopsy in some cases. The studies have shown that if the left over aspirate is preserved and processed further, it can be used for further work up of the underlying condition i.e. Immunohistochemistry and cytogenetic studies. Utilization of bone marrow clot biopsy prepared by cell block technique is a valuable technique to avoid the wastage of precious bone marrow substance aspirated from the patient[17]. The role of bone marrow clot biopsy as an adjuvant procedure in the diagnosis of diseases involving bone marrow has also been documented[18].

The present study has been planned with a view to evaluate the utility of bone marrow clot biopsy in the diagnosis of haematological and non haematological disorders.

MATERIALS AND METHODS

The present study focused upon processing and examination of bone marrow clot biopsy in different haematological disorders like anaemia, leukaemia, lymphoma, thrombocytopenia multiple myeloma, lymphoproliferative disorders, metastatic bone disease, tuberculosis and Visceral Leishmaniasis. The study was conducted over a period of 2 years from January 2018 to December 2019 at the department of Pathology Ayub Medical College Abbottabad Pakistan. A total of 335 consecutive patients referred for bone marrow examination were enrolled in the study. Children less than 3 years of age were excluded from the study because the facility for their trephine biopsy was not available in our department. After an informed written consent venous blood sample was taken for complete blood counts and blood film. Then, bone marrow sample was obtained from posterior iliac spine under local anaesthesia (2% lignocain). Smears were made from the marrow aspirate and venous blood immediately and fixed in alcohol after drying. The remaining part of bone marrow sample was allowed to clot and fixed in 10 percent buffered formol saline and processed further like soft tissue biopsy. At the same time, trephine biopsy was also obtained from the same site and fixed in 10 percent formol saline, before processing further. Slides prepared from peripheral blood were stained by May Grunewald Giemsa (MGG) and new methylene blue (for reticulocyte).Slides of bone marrow aspirate and trephine imprints were also stained by MGG stain. Pearl's staining was done on bone marrow slides for iron content. Slides of bone marrow trephine biopsy and clot biopsy were stained by haematoxylin & eosin stain after processing. Slides of peripheral blood, bone marrow aspirate and trephine imprints were examined the next day and report was prepared. These slides were kept in a rack labeled "blood and bone marrow". The slides of bone marrow clot and trephine biopsy were available for examination on day 7 and 15 respectively. They were examined independently without looking at the result of each other and that of the bone marrow aspirate. These slides were kept in separate racks labeled "bone marrow clot" and "trephine biopsy" respectively. Reports and slides of peripheral blood film, bone marrow aspirate, clot biopsy and trephine biopsy were then examined sequentially in separate sessions by double blind technique by the same pathologist to avoid bias. Final conclusive diagnosis was made on the basis of clinical information, examination of blood film, bone marrow and trephine biopsy slides. In those cases, where conclusive diagnosis could not be reached at, further tests were suggested on the study samples, in the light of microscopic findings. Results of bone marrow clot biopsy were compared with trephine biopsy to evaluate its diagnostic role. The results were expressed in tables 1 to 4 and fig 1.

Hypothesis: "There is no statistically significant difference between the results of trephine biopsy and bone marrow clot biopsy".

RESULTS

Total number of participants was 335, 169 male and 159 female with male to female ratio 1.1:1. Iron deficiency anaemia, immune thrombocytopenia and acute leukemia were among the most common diagnosis (Table1). The most common age group affected was 20 to 60 years. Trephine biopsy was positive in 326, while bone marrow clot biopsy was positive in 321 patients. Megaloblastic anaemia, normal marrow and visceral leishmaniasis were the three conditions in which clear disagreement was seen between trephine biopsy and marrow clot biopsy (Table 2). There was statistically no significant difference between the results of trephine biopsy and marrow clot biopsy, (p value >0.05, hypothesis not proved, table 3). Sensitivity, specificity and positive predictive value were high and negative predictive value was low (Table 4).

Table No.1: Demographic data and frequency distribution of disorders

S.N	Disorder	No	Age in years					Male		Female	
			Mean age 23, range 2-65					M: F = 1:1.1			
			2	20	21-40	41-60	60	No	%	No	%
1.	Iron deficiency anaemia	65	2	10	20	24	9	20	30.8	45	69.2
2.	Immune thrombocytopenia	60	10	25	15	6	4	36	60	24	40
3.	Acute Leukaemia	40	7	15	13	2	3	24	60	16	40
4.	Reactive Change	30	2	18	6	2	2	18	60	12	40
5.	Megaloblastic anaemia	25	3	8	6	4	4	15	60	10	40
6.	Myeloproliferative neoplasm	25	0	8	10	2	5	10	40	15	60
7.	Aplastic anaemia	20	2	3	10	2	3	12	60	8	40
8.	Normal Marrow	20	1	4	10	5	0	12	60	8	45
9.	Chronic Lymphocytic leukemia	14	0	0	0	6	8	8	57	6	43
10.	Lymphoma	10	0	4	2	2	2	6	60	4	40
11.	Multiple myeloma	5	0	0	0	0	5	3	60	2	40
12.	Hypersplenism	5	0	0	0	2	3	3	60	2	40
13.	Myelodysplastic syndrome	5	0	0	0	2	3	2	40	3	60
14.	Metastatic solid malignancy	4	0	0	0	2	2	2	50	2	50
15.	Granulomatous inflammation	4	0	1	3	0	0	3	66.7	1	33.3
16	Visceral Leishmaniasis	3	0	3	0	0	0	2	66.7	1	33.3
Total		335	27	99	95	61	53	176	52.5	159	47.5

Table No.2: Results of Bone Marrow Trephine biopsy versus Bone Marrow Clot biopsy

S.No	Name	No	Trephine biopsy				Bone Marrow Clot			
			Positive		Negative		Positive		Negative	
			No	%	No	%	No	%	No	%
1.	Iron deficiency anaemia	65	65	100	0	0	65	100	0	0
2.	Immune thrombocytopenia	60	60	100	0	0	60	100	0	0
3.	Acute Leukaemia	40	40	100	0	0	40	100	0	0
4.	Reactive Change	30	30	100	0	0	30	100	0	0
5.	Megaloblastic anaemia	25	22	88	3	12	22	88	3	12
6.	Myeloproliferative neoplasm	25	25	100	0	0	20	80	5	0
7.	Aplastic anaemia	20	20	100	0	0	20	100	0	0
8.	Normal Marrow	20	16	80	4	20	16	80	4	20
9.	Chronic Lymphocytic leukemia	14	14	100	0	0	14	100	0	0
10.	Lymphoma	10	10	100	0	0	10	100	0	0
11.	Multiple myeloma	5	5	100	0	0	5	100	0	0
12.	Hypersplenism	5	5	100	0	0	5	100	0	0
13.	Myelodysplastic syndrome	5	5	100	0	0	5	100	0	0
14.	Metastatic solid malignancy	4	4	100	0	0	4	100	0	0
15.	Granulomatous inflammation	4	4	100	0	0	4	100	0	0
16.	Visceral Leishmaniasis	3	1	33.3	2	66.7	1	33.3	2	66.7
Total		335	32 6	97.3	9	2.7	321	97.3	14	2.09

Table No.3: Chi square statistics of results of Bone marrow Trephine biopsy versus Clot biopsy

Test	**Diagnosed**	**Not diagnosed**	**Total**	**P-Value**
Trephine biopsy	**326**	**9**	**335**	> 0.05
Clot biopsy	**321**	**14**	**335**	

(Chi-square- 1.13, df=1, p value= 0.289, > 0.05 not significant)

Table No.4: Statistical probability of Bone Marrow Trephine and Clot biopsy (N=335)

Result	NO	
True Positive (TP)	326	
True Negative (TN)	9	
False Positive (FP)	14	
False Negative (FN)	1	
Sensitivity	326/326+14	0.96
Specificity	9/1+9	0.90
Positive predictive value	326/326+1	0.99
Negative predictive value	9/14+9	0.39

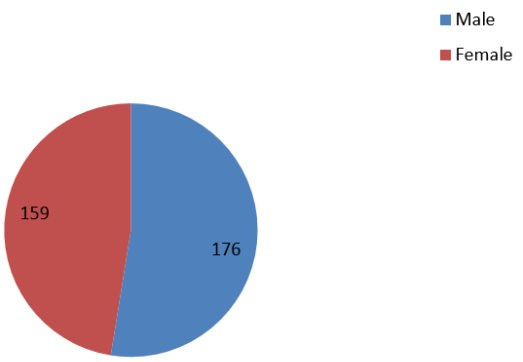

Figure No.1: Gender wise distribution of patients

DISCUSSION

Bone marrow aspirate and trephine biopsy are established diagnostic techniques since long. Role of these techniques is not fixed and varies from case to case. The important thing is that they are never interpreted separately. A conclusive opinion is made after thorough examination of the marrow aspirate and trephine biopsy and trephine imprints. Previously the usual practice was to make slides from bone marrow aspirate and discard the remaining part (also called marrow clot) of it. In the recent past, case reports and studies revealed that marrow clot may provide important clues in confirming or excluding the diagnosis, if it is processed like a soft tissue. An interesting observation was that marrow clot biopsy revealed better antigen retrieval for molecular and immunodiagnostic techniques. It can play role as adjuvant to the already established techniques of bone marrow and trephine biopsy. The present study was based upon the same hypothesis.

The present study also revealed that the diagnostic role of marrow clot biopsy is not fixed and varies from case to case, when compared with that of marrow aspirate and trephine biopsy e.g. in megaloblastic anaemia, myeloproliferative neoplasm, visceral leishmaniasis and normal marrow. A number of cases are missed which has statistical significance when looked as collective performance, despite the fact that this may not be true for individual cases like iron deficiency anaemia, immune thrombocytopenia and acute leukaemia. This is in accordance with the findings of an earlier studies[16]. In a case series Cantadori and colleagues concluded that bone marrow clot biopsy had results almost equivalent to trephine biopsy with the advantage that no decalcification was required and the result was also available earlier than that of trephine biopsy[12]. This is different from our findings. One of the reasons for this difference may be special fixation procedure adopted by the researcher which required special equipment, not available to us. Another study conducted by Ong MG and coworkers found excellent results on bone marrow clot biopsy compared with trephine biopsy and recommended it as a reliable alternative to trephine biopsy. Here again the major limitation is requirement of sophisticated processing method which is not available in every laboratory[17].

Jasim MA Al-Diab reported that as much as 81 % of the bone marrow clot section replaced the need for doing trephine biopsy. The researcher however recommended that despite good yield of bone marrow clot sections, trephine biopsy should be conducted as a recognized standard procedure. Our findings are in accordance to this study[18].

A study conducted by Toi and colleagues revealed positive correlation between bone marrow aspirate and trephine biopsy, thus confirming that two procedures are complementary[19]. The present study revealed positive correlation and complimentarity between trephine biopsy and marrow clot biopsy. Complementary nature of bone marrow aspirate, imprints of trephine biopsy and the trephine biopsy itself was also confirmed by another study. It was recommended that imprint cytology smears should be standard practice for evaluating any marrow[20]. We also suggest that in addition to bone marrow cytology, trephine biopsy and imprints, due attention should be given to marrow clot biopsy as a complementary procedure.

CONCLUSION

Clot biopsy of bone marrow plays important role in the diagnosis of many haematological and non haematological diseases. At present, it is in experimental phase and cannot replace the trephine biopsy which is a time tested diagnostic test.

Recommendations: Bone marrow clot biopsy should always be performed as a complementary test, whenever possible.

Author's Contribution:

Concept & Design of Study:	Jamila Farid
Drafting:	Muhammad Idris, Mumtaz Ahmad Khan
Data Analysis:	Nasreen Javed, Shazia Moeen
Revisiting Critically:	Jamila Farid, Muhammad Idris
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Conflict of Interest: The study has no conflict of interest to declare by any author.

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