Diagnostic Utility of Bone Marrow Aspiration and Trephine Biopsy in Neoplastic Haematological Disorders

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ABSTRACT

Introduction: Bone marrow aspiration and trephine biopsies are now widely used in the investigation and follow-up of many haematological disorders. Bone marrow biopsies are essential for the differential diagnosis of most cytopenias, myeloproliferative and lymphoproliferative disorders. Aim: Therefore present study aim is to diagnose neoplastic haematological disease through bone marrow aspiration or biopsy.

Material and methods: Present study was conducted in department of pathology, GSVM Medical College, Kanpur, on 30 patients suffering from chronic haematological disease. The relevant history of patients was recorded and informed consent was taken. Patients were investigated for complete blood count, coagulation profile, reticulocyte count and peripheral blood film (PBF) examination. Bone marrow aspiration and Bone marrow biopsy were done under aseptic precautions.

Results: Out of 30 cases, 19 (63.34%) are having neoplastic haematological diseases and most common disease is Chronic myeloid leukaemia. All cases of acute myeloid leukaemia and acute lymphocytic leukaemia were diagnosed by aspiration. In CML and multiple myeloma cases, both bone marrow aspiration and biopsy needed.

Conclusion: In cases of leukaemia and multiple myeloma, bone marrow biopsy is useful to explain the prognosis of disease. Reticulin staining further helps in explaining of prognosis. Results of aspiration and bone marrow and bone marrow biopsy are almost same, but in aspirated marrow, individual cells are perfectly preserved in well made films.

Keywords: Bone Marrow Aspiration, Neoplastic, Trephine Biopsy, Multiple Myeloma.

INTRODUCTION

Bone marrow examination (BME) is considered as valuable diagnostic tool to evaluate various haematological disorders in today's era. Indications for bone marrow examination include proper diagnosis and therapeutic monitoring of different hematological/non-hematological disorders like chronic lymphocytic leukemia, Hodgkin's and non-Hodgkin's lymphoma and multiple myeloma etc.[1-3] Bone marrow examination includes peripheral blood film (PBF), direct particle, buffy coat, bone marrow aspiration smears, trephine biopsy imprints and marrow volumetric data. Bone marrow aspirate is the sample of choice to examine nucleated red cells of marrow (myeloid: erythroid ratio). Sometimes marrow aspirate becomes diluted, dry tap, unsatisfactory eg. Metastatic deposits, focal myeloma etc. In these cases, bone marrow examination fails to demonstrate disease processes. In such cases, marrow trephine biopsy is very essential for diagnosis, by using Jamshidi needle, to improve procedure, size and quality of the specimen as compared to core needle biopsy.[4-5]

There are three ways in which marrow can be obtained that is needle aspiration, microtrephine biopsy and surgical biopsy.[6-7] Normal bone marrow is soft and semi fluid during life and consequently can be removed for examination by aspiration as well as by biopsy techniques. Improved needles permit the simultaneous performance of aspiration and biopsy.

The great value of microtrephine biopsy is that it can provide a perfect view of the structure of relatively large pieces of marrow that is, if the material obtained by biopsy, has been skilfully processed. Studies on large number of cases have been demonstrated that, whereas microtrephine biopsy specimens are superior to films of aspirated material in some circumstances, e.g. for diagnosing marrow involvement in lymphoma or non-haematological neoplastic disease.A disadvantage of most marrow trephine is that not infrequently, the specimen is crushed and its architecture altered. The Jamshidi needle[8] which has tapering end was designed to overcome this problem. Present study was done for the diagnosis of neoplastic haematological disease through bone marrow aspiration or biopsy.

MATERIAL AND METHODS

Present study was conducted on 30 patients suffering from chronic haematological disease in department of pathology, GSVM Medical College, Kanpur, These patients were admitted in LLR and associated hospitals of GSVM Medical College, Kanpur. Patients suspected of having their bone marrow involvement by any hematological or non-hematological disorders were included in study. The relevant

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history of patients was recorded and informed consent was taken. Patients were investigated for complete blood count, coagulation profile, reticulocyte count and peripheral blood film (PBF) examination. Equipments included were Sahali's apparatus, Salah's Bone marrow aspiration needle and Jamshidi's trephine biopsy needle, 5 ml and 20 ml disposable syringes, xylocaine (1%), glass slides, fixative-isopropyl alcohol, leishman's stain, Prussian blue stain and other antiseptic materials respectively. Bone marrow aspiration and Bone marrow biopsy were done under aseptic precautions. Bone marrow biopsy was taken by Zamshidi needle from the posterior iliac crest and specimen obtained is then gently removed with long probe on a slide. Obtained biopsy tissue is preserved in FAA solution, 5 ml ethyl alcohol, 10 drops acetic acid and 2 drops formalin after 6 hours fixation. The tissue is decalcified in E.D.T.A. solution, refixated in formalin and processed with paraffin-wax embedding sections, 1µm-thick, were cut and were stained by Hemotoxylin and Eosin (H&E) stain. The staining for reticulin fibers with Gomori's Silver impregnation method was done. Bone marrow biopsy and aspiration findings were analysed in context of clinical signs, symptoms and other laboratory investigations.

RESULT

Figure 1 shows that out of 30 cases, 11 (36.66%) were having non-neoplastic haematological diseases while 19 (63.34%) were from neoplastic haematological diseases.

Table 1 shows that most common neoplastic haematological disease were chronic myeloid leukaemia (36.85%) followed by acute myeloid leukaemia (21.65%).

Table 2 shows age and sex incidence in neoplastic cases. Out of 4 cases of AML, 3 were male and 1 female. Two were from 2nd decade and 2 were from 3rd decade. Out of 3 cases of ALL, 2 were male and 1 female.Both male were of 1st decade and one female was of 2nd decade. CML was the most common malignancy, out of 7 cases, 6 were male and one female. In 6 cases, 4 were of fifth decade and other 2 male were from 4th and 6th decade.

Chronic lymphocytic leukaemia was least common malignancy (only 2 cases), both were male and one each was from 5th and 7th decade. Two male and 1 female were suffering from multiple myeloma respectively from 6th and 5th decade.

Table 3 shows reticulin pattern in our cases of bone marrow biopsy. Out of 4 cases of AML, one case has normal reticulin framework and in other three, it was slightly increased. In ALL, 2 cases had slightly increased reticulin and one normal. In CML, out of 7 cases, 5 had slightly increased reticulin and 2 had normal reticulin. In CLL, one case was normal and

Disease	No. of Cases	Percentage
Acute myeloid leukaemia (AML)	4	21.65
Acute lymphoid leukaemia (ALL)	3	15.79
Chronic myeloid leukaemia (CML)	7	36.85
Chronic lymphoid leukaemia (CLL)	2	10.55
Multiple myeloma (MM)	3	15.79

Table-1: Distribution of cases according to neoplastic disease

Age group (Yrs)	AML		ALL		CML		CLL		MM	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
01-10	-	-	2	-	-	-	-	-	-	-
11-20	1	1	-	1	-	1	-	-	-	-
21-30	2	-	-	-	-	-	-	-	-	-
31-40	-	-	-	-	1	-	-	-	-	-
41-50	-	-	-	-	4	-	1	-	1	-
51-60	-	-	-	-	1	-	-	-	1	1
61-70	-	-	-	-	-	-	1	-	-	-

Table-2: Showing age and sex incidence in Neoplastic diseases

Disease	Total cases no.	Normal pattern	Increased	Decreased
Acute myeloid leukaemia	4	1	3	-
Acute lymphoid leukaemia	3	1	2	-
Chronic myeloid leukaemia	7	2	5	-
Chronic lymphoid leukaemia	2	1	-	1
Multiple myeloma	3	2	1	-

Table-3: Content of reticuline in neoplastic disease cases

Disease	Total No. Cases.	Bone marrow aspiration	Bone marrow biopsy
Acute myeloid leukaemia	4	4	-
Acute lymphoid leukaemia	3	3	-
Chronic myeloid leukaemia	7	5	2
Chronic lymphoid leukaemia	2	1	1
Multiple myeloma	3	2	1

Table-4: Comparison of bone marrow aspiration with bone marrow biopsy (Diagnostic)

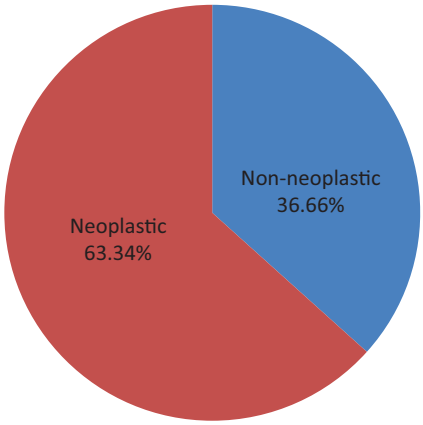

Figure-1: Distribution of cases according to disease

other one had decreased reticulin.

All 4 cases of acute myeloid leukaemia were diagnosed by aspiration. Bone marrow biopsy was least useful in these cases. Same was the case in acute lymphocytic leukaemia, in all 3 cases, bone marrow biopsy was not required as aspiration was itself conclusive. In CML, out of 7 cases, 5 were diagnosed on aspiration but in 2 cases, bone marrow biopsy was always required for diagnosis. Two cases of multiple myeloma was diagnosed by aspiration and one by bone marrow biopsy. (Table 4)

DISCUSSION

In present study, 30 patients suffering from chronic haematological disorders were included. The trephine biopsy was done to diagnose the basic cause of disease, to confirm the diagnosis of peripheral blood smear and for explaining the disease prognosis. All these patients were admitted in L.L.R and associated hospitals of G.S.V.M. Medical College, Kanpur.

In present study, in 30 cases of bone marrow biopsy, in 20 (66.66%) cases, biopsy tissue was sufficient, long enough to report while in 10 cases, we have to report on the aspiration findings. Probable reason behind that in 10 cases it was either the tissue get fractured or sometimes crushed during biopsy procedure. Similar problems reported by Inwood et al[8] who used the same needle. Cases in which biopsy was failure, it was not possible to do repeat biopsy of denial of consent by patients for this procedure and it is also advisable not to do repeat biopsy from the same site within 4-6 months.

In our study, CML was the most common malignancy, out of 7 cases, 6 were male and one female. In 6 cases, 4 were of fifth decade and other 2 male were from 4th and 6th decade. One female was 13 years of age, followed by 4 cases of AML were reported. Our observation about incidence with age and sex clearly shows male predominance of malignant disease as out of 19 neoplastic cases, only 4 were female. Burbank[9] study shows that all neoplastic diseases of haematological system are more common in males compared to females. Male to female ratio range was approximately 3:2 for acute leukaemia and 2:1 for CLL. The least degree of male to female preponderance is among children with ALL in whom the ratio is approximately 5:4. In most of the disease under consideration, the rate of increase in incidence in later decades of life is faster for male than females.

In our study, commonest age for CML was of middle age. For all commonest age was 1st and 2nd decade. AML was common in adolescence and CLL is common in elderly age. Burbank[9]study had reported that the peak of ALL was seen at the age of 3 to 4 years. ALL was quite uncommon after 40 years of age conversely is virtually unknown in persons younger than 30 years. AML and CML can occur in persons of any age but become progressively more frequent as the population reaches middle age. Out of our 19 cases of neoplastic diseases, 7 were of acute and 9 of chronic leukaemia. It is showing that incidence of acute and chronic leukaemia was equal in frequency.

The study of Burbank[9] reported that at the present, acute and chronic leukaemia appear to be equally frequent and CLL is slightly more common than CML. This study also report that CML accounts for 15% or so of leukemias and it showed slightly increasing with age. Our work differed from this study data, not on Indian secondly our study was a random one, and cases were only the small fraction of total hospital cases. One of our cases of CML was from 2nd decade (aged 13 years old girl).

Gauld et al[10] have reported that leukemia in infants and children generally is acute. Fewer than 5% of patients with CML are children.

In our study, it had been observed that a fine reticulin network exists in normalbone marrow in the interstices of which lie the haemopoietic cells. This fine network which was in continuity with the reticulin fibers of endosteum of the walls of sinusoids of the larger vessels and of that bounding fat spaces, had the appearance of a supporting stroma for the haemopoietic tissue. These findings are essentially similar to those of Howell (1891), Lowis (1891), Endrerlen (1891), Jackson (1904 and Masugi (1926).

The basic pattern of fine branching reticulin fibres of served in normal marrow was seen throughtout the whole range of haematological disorders studied. Though there was considerable quantitative variation between various group of diseases. In morphological aplasia of bone marrow, the reticulin network was very much reduced in amount. This reduction of reticulin paripassu with the number of haemopoietic cells indicate that the bone marrow reticulin is fairly labile fibre.

In the present study, it had been seen that in aplastic anaemia, there is virtually no demonstrable reticulin network. As reported by other workers, it seems that the formation of a fine reticulin network in the bone marrow is a physiological responses whereby the amount of stroma varies directly with the amount of haemopoietic tissue. In addition to the formation of this reticulin network in all types of hypercellularity of the marrow, there was at times a different pattern termed fibroblastic is described. In the present investigation this fibroblastic ppattern was seen in13 out of 30 cases. Milder degree of this change was present in one of cases of normoblastic hyperplasia and one case of hyperpleunism. It was found around all of sinusoids so it may be possible that the development of this pattern in

these instances represented a reaction to circulatory stasis or anoxia. The fibroblastic patterns was present in some cases of acute leukaemia, chronic leukaemia and multiple myeloma.

Typical megakaryocytes were observed to be markedly increased in number in only one case of our series. This case was of CML with platelet count 7.8 lac/mm^3 and also showed occulor manifestation. Increased reticulin was seen in this case.

Burston and Pinniger (1963) have reported same saying that presence of megakaryocyte like cells was always a striking feature in the cases showing a fibroblastic reticulin pattern, so that these cells be regarded as being likely to play a significant part in its formation. These workers have also concluded the findings in their study that reticulin in the marrow in haematological disorders may be decreased, normal in amount, or increased, one is an exaggeration of the normal network. Whereas other is distinctly abnormal the exaggerated normal pattern could usually be directly correlated with bulk or activity of haemopoietic tissue. The abnormal pattern was rule in clinic-pathological syndrome or myelofibrosis, but was also present to varying degrees in other haematological conditions like CML and Multiple myeloma. The abnormal pattern was usually though not invariably associated with increased fibroblastic cell activity.

Out of 30 cases, in 7 cases, diagnosis was made only by bone marrow biopsy. In 5 of these cases, it was dry tap on aspiration (3 cases of aplastic anaemia, 2 cases of CML and 1 each case of CLL & CMM).

Out of 19 cases of neoplastic diseases in making diagnosis in most of the cases, there was no significant role of bone marrow biopsy. As diagnosis can be easily made by PBS and aspiration finding except 2 cases of CML. In these two cases, we found significant fibrosis as showing poor prognosis as patients were entering into blast crisis.

Similar were the findings of Clough et al[11] he summarized that marrow examination is of little diagnostic help in CML except for the material so provided for cytogenic studies. Useful prognostic information is obtained by determining significant fibrosis is present and by studying the pattern of in vitro growth of marrow cells. The appearance of fibrosis in bone marrow has been ominous sign in patients as it was in other series.

In 4 cases of AML, diagnosis was possible by PBS and was confirmed by bone marrow aspiration. Bone marrow biopsy was useful only to evaluate that is any fibroblastic element which is increased. Manoharan[12] reported that if a biopsy has been performed, a section stained for reticulin cells will indicate some degree of myelofibrosis in 2/3rd of AML cases. Britten[13] has said that diagnostic marrow examination was unnecessary if numerous blast cells are present in PBS. Biopsy should only be performed in cases in which acellular particles are present in aspiration. In case of ALL, we found two L-2 variant of disease and one of L-3. On marrow aspiration, significant material was available and blast was predominant cells. Bone marrow biopsy was of little significance.

Fahey et al[15] reported that bone marrow biopsy is not necessary or particularly less useful in the diagnosis of ALL. It commonly reveals hypercellular marrow. It will also disclose increased fibrosis in majority of patients similar was the case in CLL. Biopsy was required only to differentiate with non-Hodgking lymphoma.

Out of 3 cases of multiple myeloma, bone marrow biopsy was significant in only one case in which material by aspiration was inconclusive. The diagnosis of multiple myeloma was easily possible with PBS and bone marrow aspiration with X-Ray findings.

Vilter et al reported that when a patient thought to have aplastic anaemia is found to have a normally cellular or hypercellular marrow have a normally cellular or hypercellular marrow. One explanation for such a contradictory finding is that the biopsy needle has entered an area in which bone marrow is regenerating after severe damage. Another not uncommon dilemma a patient thought to have leukemia. In most situations a larger marrow sample obtained by biopsy will solve the problem.

et al reported that in high number of cases on bone marrow aspirate there was dry tap or chiefly mature red cells seen in aplastic anaemia. Such finding makes it advisable to obtain a larger specimen, by biopsy to be sure that one has obtained true bone marrow and not blood, and also to see how fatty the marrow is. However, the most important bone marrow finding is the proportion of cells that are non-myeloid and not erythroblasts since the proportion of such cells has been found to be directly to mortality. Hypocellular marrow, leukoerythroblastic blood picture and marrow fibrosis are strong indications for Bone marrow biopsy.[15]

CONCLUSION

From above results we conclude that chronic myeloid leukaemia is the most common malignant haematological disease. In cases of leukaemia and multiple myeloma, bone marrow biopsy is useful to explain the prognosis of disease. Reticulin staining further helps in explaining of prognosis. Results of aspiration and bone marrow and bone marrow biopsy are almost same, but in aspirated marrow, individual cells are perfectly preserved in well made films.

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