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STUDY OF DIAGNOSTIC EFFICACY OF BONE MARROW CLOT SECTION IN CYTOPENIA CASES.

Pathology

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ABSTRACT

Introduction: Bone marrow examination has become an indispensable adjuvant to diagnose hematological disorders. For the last few years, some studies evaluated applicability of clot sections in diagnosis and follow-up in several diseases. When both the procedures for bone marrow examination i.e., aspiration (BMA) and clot section (BMC) are done simultaneously, they can improve the diagnostic yield and are helpful for early accurate diagnosis in cytopenia cases. The present study was done with the aim to assess the diagnostic accuracy of clot section and compare with that of aspiration. **Materials and Methods:** The present study was carried out on 144 cytopenia cases. Cell block were prepared from the clot that remained after preparation of BMA smears; and BMC were examined for cellularity as well as morphology. Trephine biopsy (Bm Bx) was done in 81 cases. **Results:** Of the total 144 cytopenia cases, BMA was diagnostic in 124 (86.1%) cases, while BMC was diagnostic in 131 (90.9%) cases. p value was found to be significant (0.00001%). BM Bx was diagnostic in all 81 (100%) cases, of which BMC was diagnostic in 69 (85.2%) cases. **Conclusions:** BMA and BMC done simultaneously provided more material and enabled us to study the cytomorphology of the cells. BMC was found to be easy, rapid and complementary technique along with BMA. BMC was particularly useful in the detection of marrow lesions with focal distribution (particularly in a case of TB). The advantage of both the procedures done together increases the diagnostic yield.

KEYWORDS

Bone marrow aspiration, clot section, trephine biopsy, diagnostic accuracy.

INTRODUCTION

Bone marrow examination is valuable in the diagnosis of number of hematological conditions like anemia, thrombocytopenia, leukemia, myeloproliferative neoplasms, as well as non-hematological conditions like tuberculosis, pyrexia of unknown origin, metastatic cancers, osteoporosis [1]. Bone marrow evaluation is not only essential for continuous assessment of the underlying pathology, but also for special tests like flowcytometry, cytogenetics and molecular profile [2]. Bone marrow examination includes Bone marrow aspirate (BMA), bone marrow biopsy (BM Bx), touch imprints and Clot section (BMC) [3].

BMA and imprint smears are used for qualitative and quantitative cytological assessment of marrow cells, primarily by differential count, myeloid erythroid (M: E) ratio, maturation status and morphological details [4]. In addition, biopsy is usually performed to study architecture of marrow and to look for cellularity, fibrosis in conditions like lymphoma, myeloma, metastatic tumour or granulomas, where the involvement can be focal [5,6].

The aspirated material left behind, after making adequate number of slides, is usually discarded; although it can be used to prepare a clot section, similar to cell block that is extensively used in cytological practices. Clot section allows histological evaluation to supplement cytological analysis and broadens BM exam sensitivity [7].

Clot technique is not being frequently utilized in routine clinical practice though it has been described in literature [8]. For the last few years, some studies evaluated its applicability in diagnosis and follow-up in several hematological diseases [9]. It has been observed that if aspirate is allowed to clot and processed like a soft tissue biopsy histopathology, it may provide valuable information and complement the final diagnosis [10].

Clot sections provide a better and more detailed estimate of bone marrow cellularity. They are particularly useful for patients with aplastic / hypoplastic marrow [4]. Clot sections can be used for further work up of the underlying condition with help of Immunohistochemistry [7].

The present study was done on cytopenia cases. Clinical findings and hematological parameters were evaluated. Diagnostic efficacy of BMC was assessed and compared with that of bone marrow aspiration. Biopsy were done whenever needed and taken as gold standard.

MATERIALS AND METHODS

The present cross sectional prospective study was carried out in the department of Pathology in a tertiary care hospital in Central India, over a period of 2 years.

The cytopenia cases were selected on the basis of clinical features and laboratory results. BMA, BMC and BM Bx were carried out from posterior superior iliac spine, after getting a written consent from the patient or the guardian. Indoor patients with presence of any two or all three (ie. Hemoglobin < 10 gm/dl, Total leukocyte count (TLC) < 4000/cumm, Platelet count < 1,50,000/cumm) on CBC and peripheral smear examination were included in study.

The cases with bleeding disorders, INR >1.5 and dry tap on BMA were excluded from the study. Samples were subjected to CBC, Retic count, Peripheral smear and Bone marrow examination. After preparing 4-6 smears from bone marrow aspirate; remaining aspirate was kept on slides and allowed to form clot, which was fixed in 10% formalin and was processed like small biopsies in histopathology [10]. Sections were made from clot and stained with H&E stain, or other special stain as per requirement.

Other biochemical investigations were done as per clinical history; like serum Triglyceride, Fibrinogen, Ferritin, C-reactive protein, Alkaline Phosphatase, Prothrombin Time, Lactate dehydrogenase, D-dimer, etc.

RESULTS

Out of total 1200 bone marrow examinations performed in 2 years; 144 cases who presented with cytopenia were studied. There were 99 (68.75%) cases of pancytopenia and 45 (31.25%) cases of bicytopenia.

Amongst the total 144 cases, the most common clinical finding was pallor seen in 133 (92.4%) cases, followed by weakness in 107 (74%), fever in 79 (54.9%), weight loss in 44 (30.5%), splenomegaly in 36 (25%), breathlessness in 30 (21%), bleeding in 29 (20%), hepatomegaly in 13 (9%) and lymphadenopathy in 8 (5.6%) cases. Individual cases had more than one clinical finding.

Anemia was the commonest hematological disorder seen in 79 (54.9%); followed by 23 (15.9%) cases of infection; 16 (11.1%) cases of peripheral destruction of platelets; 8 (5.6%) cases of hypersplenism

and leukemia each; 5 (3.5%) cases of Myelodysplastic syndrome; 3 (2.08%) cases of Myelofibrosis and 2 (1.4%) cases of metastatic deposits**.**

Of the total 79 cases of anemia, there were 34 (23.6%) cases of aplastic anemia, 33 (22.9%) of megaloblastic anemia, 10 (6.9%) of dimorphic anemia and 2 (1.4%) of pure red cell aplasia. Amongst the 23 (15.9%) cases of infection, 13 (9.03%) cases were of Hemophagocytic lymphohistiocytosis (HLH), 9 (6.25%) were of reactive changes associated with infection / inflammation and 1 (0.7%) case was of Tuberculosis.

Distribution of hematological disorders along with their demographic data and hematological parameters of 144 cytopenia cases are given in **Table 1**.

Of the total 144 cases, BMA was diagnostic in 124 (86.1%) and inconclusive in 20 (13.9%) cases; while BMC was diagnostic in 131 (90.9%) and inconclusive in 13 (9.1%) cases**.** p value was found to be significant (0.00001) i.e., < 0.05.

Bone marrow Biopsy (BM Bx) was done in 81 out of total 144 cases of cytopenia. Of these 81 cases, BMC was diagnostic in 69 (85.2%) and inconclusive in 12 (14.8%) cases; while BM Bx was diagnostic in all 81 (100%) cases.

Diagnostic evaluation of BMA, BMC and BM Bx in Cytopenia Cases is given in **Table 2**.

Bone marrow clot section (BMC) findings of Aplastic anemia, Tuberculosis, Peripheral destruction of platelets and Myelofibrosis are shown in **Figure 1, Figure 2, Figure 3** and **Figure 4** respectively.

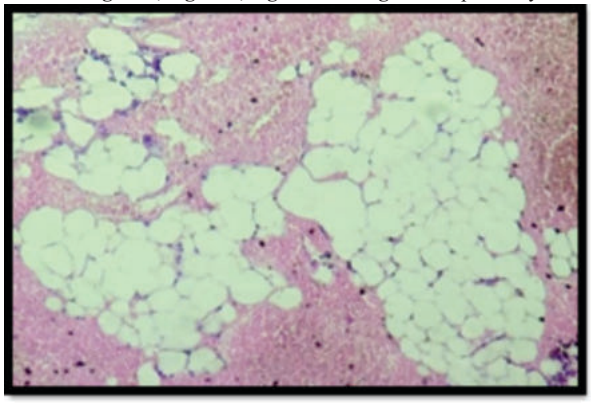

Figure 1: BMC showing increased fat in Aplastic anemia case with trilineage suppression.

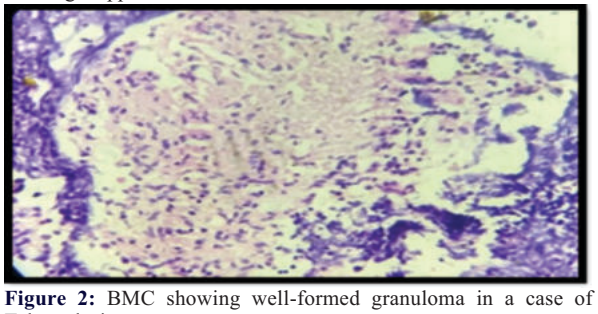

Figure 2: BMC showing well-formed granuloma in a case of Tuberculosis.

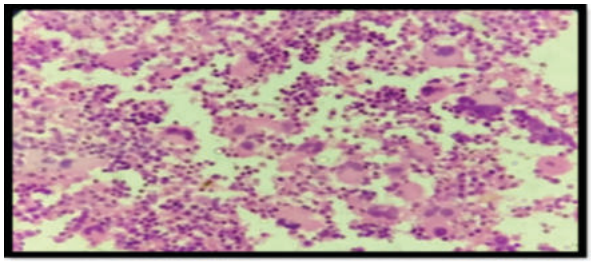

Figure 3: BMC showing increased megakaryocytes in a case of Peripheral destruction of platelets.

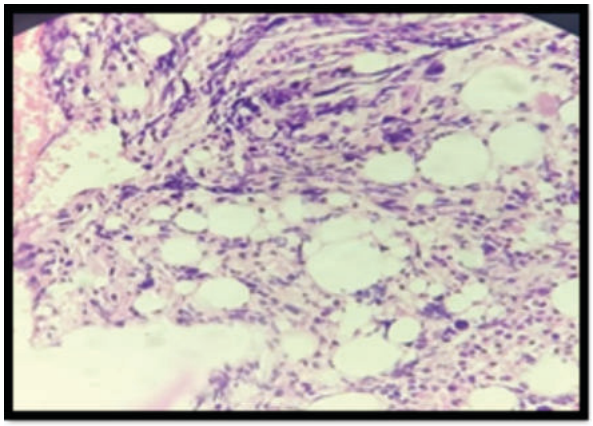

Figure 4: BMC showing increased fibrosis in a case of Myelofibrosis.

Table 1: Distribution Of Hematological Disorders With Demographic Data And Hematological Parameters In Cytopenia Cases (n=144).

Sr. No.	Distribution of Hematological Disorders (n=144)	Mean age (yrs)	Age range (yrs)	Most common Age groups (yrs)	M:F ratio	Hb range (gm/dl)	TLC range (/cumm)	Platelet range (/cumm)
1	Aplastic Anemia (AA) 34 (23.6%)	36	2 - 70	1 – 20	1 : 1	3 – 10	600 – 4000	5000 – 1,45,000
	Megaloblastic Anemia (MA) 33 (22.9%)	40.5	6 - 75	11 – 30	1.4 : 1	1.6 - 9.7	1300 – 9600	13000 – 283000
	Dimorphic Anemia (DA) 10 (6.9%)	37.5	14 - 61	11 – 30	1.5 : 1	1.4 - 9.6	900 – 8000	10,000 – 2,90,000
	PRCA 2 (1.4%)	7.5	5 - 10	1 – 10	1 : 1	1.5 - 4.7	3500 – 3800	1,02,000 – 2,40,000
2	Infections 23 (15.9%)	35	2 months – 70 yrs	1 – 20	1 : 1.9	1.8 – 10	800 - 12,000	11,000 - 1,33,000
3	Peripheral Destruction of Platelets (PDP) 16 (11.1%)	35.5	11 - 60	11 – 30	1 : 2.2	3.4 - 10.7	3000 – 12000	4000 – 76000
4	Leukemia 8 (5.6%)	27	4 - 50	1 – 20	3 : 1	2.7 - 9.9	4200 - 26,000	18,000 - 70,000
5	Hypersplenism (HS) 8 (5.6%)	29.5	14 - 45	11 – 30	1 : 7	1.3 - 9.7	1000 – 4000	20000 –1,18000
6	MDS 5 (3.5%)	62.5	43 - 82	61 – 70	1.5 : 1	2.3 – 6	2100 – 5000	12,000 - 55,000
7	Myelofibrosis (MF) 3 (2.08%)	52	49 - 55	41 – 60	1 : 2	6.5 - 9.1	2620 – 9200	8,000 – 73,000
8	Metastatic Deposits 2 (1.4%)	61.5	57 - 66	51 - 70	1 : 1	5.6 – 7.6	7000 – 10000	21000 – 84000

Total 144 (100 %)	41	2 months – 82 yrs	11 - 30	1 : 1.14	1.3 – 10.7	600 – 26,000	4000 – 2,90,000

Table 2: Diagnostic evaluation of BMA, BMC and BM Bx in Cytopenia Cases.

Sr. No.	Disorders (n= 144)	BMA (n= 144)		BMC (n= 144)		BM Bx (n= 81)	
		Diagnostic	Inconclusive	Diagnostic	Inconclusive	Diagnostic	Inconclusive
1	Aplastic Anaemia (AA) 34 (23.6%)	17 (50%)	17 (50%)	25 (73.52 %)	09 (26.47%)	34 (100%)	0
	Megaloblastic Anaemia 33 (22.9%)	33 (100%)	0	33 (100%)	0	04 (100%)	0
	Dimorphic Anaemia 10 (6.9%)	10 (100%)	0	09 (90%)	01 (10%)	03 (100%)	0
	PRCA 2 (1.4%)	2 (100%)	0	2 (100%)	0	2 (100%)	0
2	Infections 23 (15.9%)	22 (95.7%)	1 (4.3%)	23 (100%)	0	0	0
3	Peripheral Destruction of Platelet (PDP) 16 (11.1%)	16 (100%)	0	16 (100%)	0	14 (100%)	0
4	Leukemia 8 (5.6%)	8 (100%)	0	8 (100%)	0	6 (100%)	0
5	Hypersplenism (HS) 8 (5.6%)	8 (100%)	0	8 (100%)	0	8 (100%)	0
6	MDS 5 (3.5%)	4 (80%)	1 (20%)	4 (80%)	1 (20%)	5 (100%)	0
7	Myelofibrosis (MF) 3 (2.08%)	2 (67%)	1 (33%)	1 (33%)	2 (67%)	3 (100%)	0
8	Metastatic Deposits 2 (1.4%)	2 (100%)	0	2 (100%)	0	2 (100%)	0
Total 144 (100%)		124 (86.1%)	20 (13.9%)	131 (90.9%)	13 (9.1%)	81 (100%)	0

DISCUSSION

Clinical finding of pancytopenia is common and is seen in many hematological diseases, which remains a challenge for both clinicians and pathologists. Bone marrow examination is very crucial investigation in patients of pancytopenia [11].

In the recent past, marrow clot has been studied and has been evaluated as an additional diagnostic method for diseases affecting marrow[7]. In the present study, diagnostic role of BMC is studied and compared with BMA and BM Bx in various neoplastic and non-neoplastic hematological conditions.

Of the 34 Aplastic anemia cases, BMA was diagnostic in 17 (50%) cases; whereas BMC was diagnostic in 25 (73.5%) cases. The 8 discordant cases showed diluted BMA; while BMC favored AA, which were confirmed on BM Bx. **Jamila et al** [7] found BM clot diagnostic in all 100 % cases of AA.

In Megaloblastic anemia, both BMA and BMC were diagnostic in all 33 (100%) cases. **Jamila et al** [7] study found BMC diagnostic in 88 % cases.

Of the 10 Dimorphic anemia cases, BMA was diagnostic in all 10 (100%) cases; while BMC was diagnostic in 9 (90%) cases. The 1 discordant case was diluted on BMC, which was later confirmed by BM Bx. DA was not found in the studies on clot sections by various other authors.

In one case of TB, BMA was diluted and failed to diagnose; whereas clot section showed well- formed caseating granuloma which was positive for AFB by ZN stain. Study by **Jamila et al** [7] found 100% cases of granulomatous infection diagnosed on BMC.

BMA and BMC were diagnostic in all 16 (100%) cases of PDP. **Jamila et al** [7] also found ITP diagnostic on BMC in 100% cases. Megakaryocytes and precursors are better appreciated by H & E section as suggested by the study by **Uniya et al** [4].

BMA and BMC were diagnostic in all the 8 (100%) cases of Leukemia in present study; which is comparable with the study of **Jamila et al** [7] who also diagnosed acute leukemia on BMC in 100% cases.

In Hypersplenism cases, BMC was diagnostic in all 8 (100%) cases; which correlated well with **Jamila F et al** [7] **and U. Uniya et al** [4] who also found clot section diagnostic in 100 % cases of hypersplenism.

Of the 5 MDS cases, BMA and BMC were diagnostic in 4 (80%) and inconclusive in 1 (20%) case. In this 1 inconclusive case, aspiration as well as clot section were diluted; while BM Bx confirmed the diagnosis of MDS. **Jamila et al** [7] found 100 % MDS cases diagnostic on BMC.

Out of 3 MF cases, BMA was diagnostic in 2 (67%) cases; while BMC was diagnostic in 1 (33%) case only. BMA was diluted in 1 (33%) case; while BMC was diluted in 2 (67%) cases. BM Bx was diagnostic in all the 3 (100%) cases.

In present study BMA and BMC were diagnostic in both (100%) cases of metastatic deposits; which correlated well with the study of **Jamila et al** [7] who also found 100 % cases diagnostic on BMC.

In present study, the diagnostic accuracy of BMC was more (90.9 %) than that of BMA (86.1%). **Jasim A. et al** [12] and **Vidya et al** [8] in their studies found the diagnostic accuracy of BMA was more than that of BMC.

In present study, BM Bx was done in 81 cases of cytopenia. The diagnostic accuracy of BMC (when compared with BM Bx) was found to be 85.2%; while that of BM Bx was 100%. Our study correlated well with the study of **Jamila et al** [7] who also found the diagnostic accuracy of BM Bx more (97.3%) than that of BMC (95.8%).

Simultaneous evaluation of both BMA and BMC can increase the diagnostic yield in cases of cytopenia [13]. p value was statistically significant (0.00001) in the present study. It is therefore recommended to perform BMC as an adjuvant procedure along with BMA and BM Bx to increase the diagnostic yield [12,14].

In study by **Cantadori et al** [9], clot biopsy showed equivalent results to trephine biopsy and results of clot biopsy were available earlier with an advantage that no decalcification was required. These findings were comparable to the present study.

CONCLUSION

In present study we found BMC a complementary technique along with BMA. Morphological features on BMC were almost similar to that of BM Bx and helped in early diagnosis, as no decalcification required.

Detailed primary hematological investigations along with BMA, BMC and BM Bx are helpful for understanding of the disease process, to diagnose or to rule out the causes of cytopenia and helpful in planning further investigations and management of cytopenia patients**.**

BMC is useful in cases with diluted aspirate or inadequate biopsy, blocks of BMC can be preserved for longer duration and multiple slides can be prepared. IHC and special stains can also be done. However, BMC cannot replace trephine biopsy, which is a time-tested diagnostic test.

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