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Original Article

Pathology Section

Diagnostic Utility of Bone Marrow Aspiration, Trephine Biopsy, and Flow Cytometry in the Evaluation of Various Haematological and Non Haematological Disorders: A Cross-sectional Study from Northern India

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ABSTRACT

Introduction: The spectrum of haematological and non haematological disorders is vast in various age groups. Complete blood counts and other routine laboratory tests are not always sufficient to diagnose these diseases. Bone marrow examination plays an important role in diagnosing their underlying causes.

Aim: To analyse the spectrum of various haematological and non haematological disorders reported in Bone Marrow Aspiration (BMA) and compare them with Bone Marrow Trephine Biopsy (BMB) and Flow Cytometry (FCM) findings where applicable.

Materials and Methods: A one-year cross-sectional study was conducted in the Department of Clinical Pathology, PGIMS, Rohtak, Haryana, India from April 2022 to March 2023. A total of 518 consecutive BMA samples were morphologically analysed. Comparative evaluations were performed among BMA, BMB, and FCM where applicable. Diagnostic accuracy was calculated, and the findings of discordant cases were tabulated. Definitive diagnosis of lymphoma/leukaemia and Paroxysmal Nocturnal Haemoglobinuria (PNH) cases were made using FCM.

Results: The patients' ages ranged from three months to 86 years, with a mean age of 38.4 years. The male to female ratio was 1.3:1, with a slight male predominance. The highest number of cases were of anaemia {183 (35.3%) and 164 (31.7%), respectively} and leukaemia {128 (24.7%) and 134 (25.9%), respectively}, followed by normal marrow studies {39 (7.5%) and 32 (6.2%), respectively} and megakaryocytic thrombocytopenia {24 (4.6% in each} in both BMA and biopsy. Among anaemia cases {183 and 164 cases in BMA and BMB}, the majority were of the megaloblastic type {62 (33.8%) and 54 (32.9%), respectively}, followed by hypoplastic/aplastic type {40 (21.8%) and 50 (30.5%), respectively}. In cases of leukaemia (128 and 134 cases in BMA and BMB), acute leukaemia cases (76 (59.4%) and 82 (61.2%), respectively} outnumbered chronic leukaemia cases {52 (40.6%) and 52 (38.8%), respectively} in both BMA and BMB. The concordance and discordance rate between BMA and BMB were 87.6% (419 cases) and 12.4% (59 cases), respectively. Diagnosis was exclusively made by BMB in cases of myelofibrosis, granulomatous disease, and Hodgkin's lymphoma.

Conclusion: BMA cytology is a relatively safe and mildly invasive technique for evaluating various haematological and non haematological disorders with better preservation of cellular morphology. However, in cases with dry/blood taps and focal marrow involvement, BMB should be performed, as it shows well-preserved marrow architecture with all its cellular and stromal components. FCM is a definitive diagnostic modality for further categorisation of acute leukaemia and Chronic Lymphoproliferative Disorders (CLPD).

Keywords: Anaemia, Leukaemia, Lymphoma, Megakaryocytic thrombocytopenia, Paroxysmal nocturnal haemoglobinuria

INTRODUCTION

Bone marrow is involved in variety of haematological and non haematological disorders. Haematological disorders include chronic anaemia, pancytopenia, aplastic anaemia, thrombocytopenic purpura, hypersplenism, acute leukaemia, Myeloproliferative Neoplasm (MPN), and haematolymphoid neoplasms. Non haematological disorders encompass infectious diseases that infiltrate the bone marrow, such as tuberculosis, parasitic infections, and metastatic deposits. Bone marrow examination is an important tool that aids in the diagnosis and management of these disorders [1]. BMA and BMB are complementary to each other. The aspirate smears are useful for studying the morphology of cells and for obtaining a differential cell count. They are also valuable for additional flow cytometric, immunophenotyping, cytogenetic, and molecular studies. However, BMB is of value when bone marrow aspirate yields a dry tap or a blood tap as it provides information on architecture, cellularity, fibrosis, and the pattern of distribution of abnormal infiltrates [2].

The diagnostic utility of both modalities differs in different conditions. Simultaneous assessment of BMA and BMB allows for a more detailed marrow assessment that may be impossible to achieve with the use of any one approach alone. Although both procedures are performed simultaneously, they are assessed at different points in time. Pathologists often view the BMA smears in the clinical pathology section in isolation from the BMB as it is processed rapidly. The BMB is received in the histopathology section, and its processing is a lengthy and tedious procedure as it requires decalcification; additionally, histopathologists may not have steady access to the aspirate smears. While results are often concordant, discordance can occur. This discordance can lead to perplexity about the diagnosis and delays in treatment [3]. FCM is a modality with increasing application in modern haematology practice. This is due to the rapidity of obtaining results, ease of use, and increasing power to detect abnormal populations of cells. Flow cytometric immunophenotyping is an accurate method for the quantitative and qualitative evaluation of haematopoietic cells. Its major uses in malignant haematology are in the diagnosis, classification, and monitoring of diseases such as leukaemia, lymphoma, and myeloma. The technique is now also used to detect disease-specific populations of cells in PNH [4].

The aim of this study was to analyse the spectrum of various haematological and non haematological disorders reported on BMA and compare them with BMB and FCM findings wherever applicable to formulate an effective and rapid method for diagnosing a wide spectrum of diseases. This study will highlight the diagnostic utility of BMA, BMB, and FCM in various haematological and non haematological disorders, as combined analyses are useful in achieving more accurate and informative data in some diagnostically challenging cases.

MATERIALS AND METHODS

A retrospective cross-sectional study was conducted on consecutive 518 BMA samples received in the Department of Clinical Pathology, Pt. BD Sharma, PGIMS Rohtak, Haryana, over a period of one year from April 2022 to March 2023.

Inclusion criteria: All consecutive BMA samples were included in the study.

Exclusion criteria: Inadequate BMA and trephine biopsies were excluded from the study.

Clinical analysis: Detailed clinical history and results of previous investigations were obtained from all cases. A 2 mL Ethylenediaminetetraacetic Acid (EDTA) blood sample was collected for complete blood counts and reticulocyte count using BC-6800 MINDRAY and for peripheral blood film examination. BMA and biopsy were performed from the same site using a one-needle technique under local anaesthesia with a lignocaine solution. The Posterior Superior Iliac Spine (PSIS) was the preferred site, while the sternum was used as the aspiration site in obese patients.

Morphological assessment: Aspiration and imprint smears were air-dried and stained with Leishman-Giemsa (LG) stain. Cytochemical stains like Periodic Acid Schiff (PAS), Myeloperoxidase (MPO), and Sudan black were conducted in cases of haematological malignancies, and Perl's Prussian blue was used for assessing iron stores in cases of anaemia. The biopsy specimen was fixed in 10% neutral buffered formalin and subjected to decalcification in a 5.5% EDTA solution for 72 hours. Subsequently, the length of the biopsy was noted, ranging from 0.8 to 1.5 cm, and it underwent routine processing in an automated tissue processor before being embedded in paraffin. Sections 2-3 µm thick were cut and stained with haematoxylin and eosin. Reticulin and Masson's trichrome stains were performed to grade bone marrow fibrosis. Immunohistochemistry (IHC) was conducted using the standard streptavidin-biotinylated peroxidase method.

Flow cytometry analysis: Immunophenotyping was performed on an eight-colour flow cytometer BD FACS Canto II (Becton Dickinson, San Jose, CA) using a monoclonal antibody panel for acute leukaemia, Chronic Lymphoproliferative Disorders (CLPD), and paroxysmal nocturnal PNH on peripheral blood/bone marrow samples.

Common antibodies used in the acute leukaemia panel included CD34, HLA-DR, terminal Deoxynucleotidyl Transferase (TdT), myeloid markers (cMPO, CD13, CD33, CD117), monocytic markers (CD64), B lymphoid markers (CD19, CD10, CD20, cCD79a), and T lymphoid markers (CD3, CD5, CD7, CD4, CD8). Antibodies used in the CLPD panel were CD3, CD5, CD19, CD20, CD23, FMC7, CD10, kappa, lambda, CD25, CD103, CD38, CD7, CD4, CD8. For PNH, gating antibodies used were CD45, CD15, CD64, GPI-linked antibodies CD59, CD14, CD24, and Fluorescent Aerolysin (FLAER). All standard protocols were followed.

STATISTICAL ANALYSIS

The BMA, BMB, and FCM were reported by different pathologists and were blinded to each other's reports. The reports of each case were reviewed and compared, and the diagnostic accuracy was calculated. The findings of discordant or inconclusive cases and the reasons for discordance were tabulated. The results were then statistically analysed using the Statistical Package for the Social Sciences (SPSS) version 20.0 (IBM Corp., SPSS Statistics, Armonk, NY) for Windows.

RESULTS

In the present study, a total of 518 cases subjected to BMA over a period of one year were analysed retrospectively. Out of the 518 BMA samples, 52 were deemed unsatisfactory due to aparticulate, haemodiluted marrow smears (44 cases) and dry tap (08 cases). Bone marrow BMB was not performed in 44 cases and was inadequate in 15 cases due to insufficient biopsy length or the presence of only cartilage, cortical bone, or a blood clot.

Age and sex distribution: The age of the patients ranged from three months to 86 years, with a mean age of 38.4 years. The majority of patients were in the age range of 21 to 30 years, accounting for 96 (18.5%), followed by the 11 to 20 years age group, with 88 (16.9%). Out of the 518 cases, 294 (56.8%) were males and 224 (43.2%) were females, forming a ratio of 1.3:1, indicating a slight male preponderance [Table/Fig-1].

Age group (years)	Male, n (%)	Female, n (%)	Total no. of cases (%)
0-10	26 (59.1)	18 (40.9)	44 (8.5)
11-20	48 (54.5)	40 (45.5)	88 (16.9)
21-30	50 (52.1)	46 (47.9)	96 (18.5)
31-40	36 (62.1)	22 (37.9)	58 (11.2)
41-50	34 (59.6)	23 (40.4)	57 (11.1)
51-60	40 (57.2)	30 (42.8)	70 (13.5)
61-70	32 (57.2)	24 (42.8)	56 (10.8)
71-80	26 (56.5)	20 (43.5)	46 (8.9)
81-90	02 (66.7)	01 (33.3)	03 (0.6)
Total	**294 (56.8)**	**224 (43.2)**	**518 (100)**

[Table/Fig-1]: Age and sex distribution.

Clinical and laboratory indications for bone marrow examination: The most common indication was anaemia under evaluation 161 (31%), followed by suspected malignancy 104 (20%), and pancytopenia 67 (13%). Other indications included fever/pyrexia of unknown origin, organomegaly (hepatomegaly/splenomegaly/lymphadenopathy), leukopenia, thrombocytopenia, and monitoring of therapy/follow-up of leukaemia/lymphoma patients [Table/Fig-2].

Indications of bone marrow examination	n (%)
Anaemia under evaluation	161 (31)
Suspected malignancy	104 (20)
Pancytopenia	67 (13)
Fever	62 (12)
Organomegaly	52 (10)
Leukopenia	36 (7)
Thrombocytopenia	26 (5)
Monitoring therapy	10 (2)

[Table/Fig-2]: Various indications for bone marrow examination.

Spectrum of haematological and non haematological disorders on BMA and BMB: Patients were diagnosed on the basis of bone marrow examination. In both BMA and BMB, the maximum number of cases were of anaemia 183 (35.3%) and 164 (31.7%), respectively and leukaemia 128 (24.7%) and 134 (25.9%), respectively, followed by a normal marrow study 39 (7.5%) and 32 (6.2%), respectively and megakaryocytic thrombocytopenia 24 (4.6%) in each [Table/Fig-3].

Among cases of anaemia (183 and 164 cases in BMA and BMB), the majority were of the megaloblastic type 62 (33.8%) and 54 (32.9%), respectively, followed by hypoplastic/aplastic type 40 (21.8%) and 50 (30.5%), respectively. Among cases of leukaemia (128 and 134 cases in BMA and BMB), acute leukaemia cases 76 (59.4%)

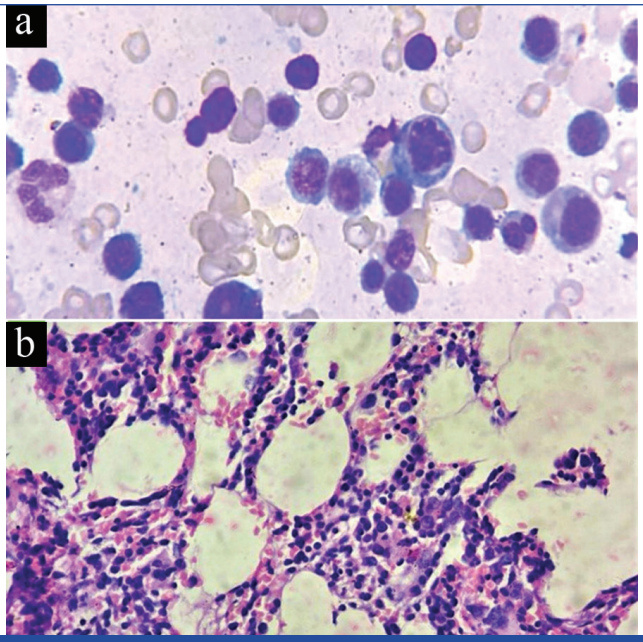
S. No.	Disorders	Bone Marrow Aspiration (BMA)	Bone Marrow Trephine Biopsy	Diagnostic accuracy (%)
1.	**Anaemia**			
	Megaloblastic	62	54	*
	Hypoplastic/Aplastic	40	50	80
	Normoblastic	18	12	*
	Micronormoblastic	29	22	*
	Dimorphic	34	26	*
	Total	**183**	**164**	
2.	**Leukaemia**			
	Acute leukaemia	18	06	#
	Acute myeloid leukaemia	26	34	76.4
	Acute lymphoblastic leukaemia	32	42	76.2
	Chronic myeloid leukaemia			
	• Chronic phase	08	08	100
	• Accelerated phase	06	05	#
	• Blast phase	20	21	95.2
	Chronic lymphocytic leukaemia/Chronic Lymphoproliferative Disorder (CLPD)	18	18	100
	Total	**128**	**134**	
3.	**Lymphoma**			
	Non Hodgkin Lymphoma	06	10	60
	Hodgkin Lymphoma	00	02	--
4.	Marrow in remission for leukaemia	10	10	100
5.	Myelofibrosis	00	06	--
6.	Myeloproliferative disorder	13	16	81.2
7.	Myelodysplastic syndrome	03	06	50
8.	Plasma cell dyscrasia	12	14	85.7
9.	Megakaryocytic thrombocytopenia	24	24	100
10.	Non specific myeloid reaction	22	18	*
11.	Lymphoplasmacytosis	16	09	*
12.	Eosinophilic reaction	08	08	100
13.	Granulomatous disease	00	03	--
14.	Leishmaniasis	01	01	100
15.	Metastasis	01	02	50
16.	Normal marrow study	39	32	#
17.	Aparticulate haemodiluted	44	--	--
18.	Dry tap	08	--	--
19.	Inadequate	--	15	--
20.	Not performed	--	44	--
	Total	**518**	**518**	

[Table/Fig-3]: Comparative evaluation of Bone Marrow Aspiration (BMA) and trephine biopsy diagnosis.
*In some of the cases of anaemia (29 cases), lymphoplasmacytosis (7 cases) and non specific myeloid reaction (4 cases) trephine biopsy was not performed especially in children <12 years of age. Hence, diagnostic accuracy was not calculated in such cases (7.7%)
Values are missing at places where either BMA or BMB was done as in such cases diagnostic accuracy cannot be calculated; #These cases were re-classified on BMB after application of IHC

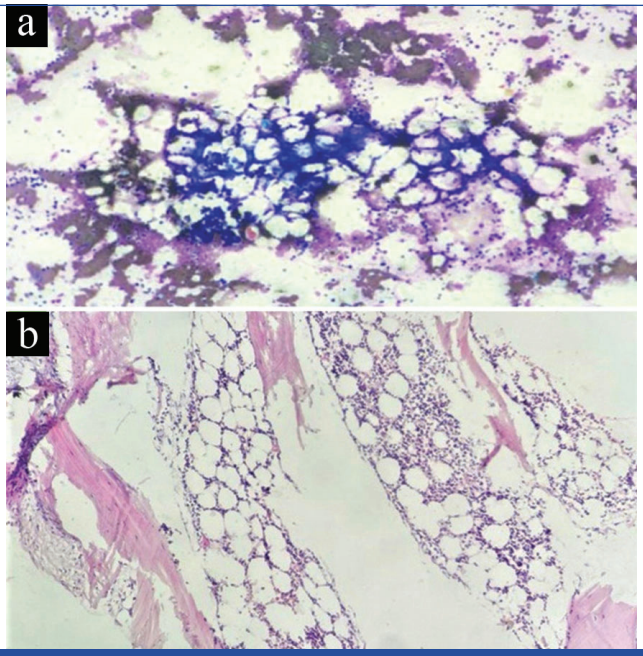
and 82 (61.2%), respectively outnumbered chronic leukaemia cases 52 (40.6%) and 52 (38.8%), respectively in both BMA and BMB [Table/Fig-4-6].

Out of the total 518 cases, both BMA and BMB were performed in 478 cases, out of which concordance was seen in 419 (87.6%) and discordance was seen in 59 (12.4%).

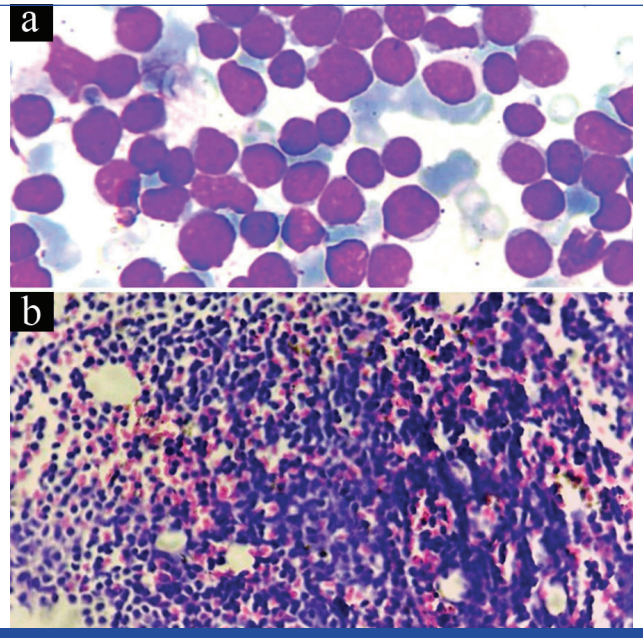
Diagnostic accuracy was 100% in the case of CML-CP, CLPD, megakaryocytic thrombocytopenia, eosinophilic reaction, and remission marrow for leukaemia. However, in some cases of anaemia



[Table/Fig-4]: Megaloblastic anaemia: a) BMA LG, X400; b) BMB H&E, X400.



[Table/Fig-5]: Hypoplastic marrow: a) BMA LG, X200; b) BMB H&E, X200.

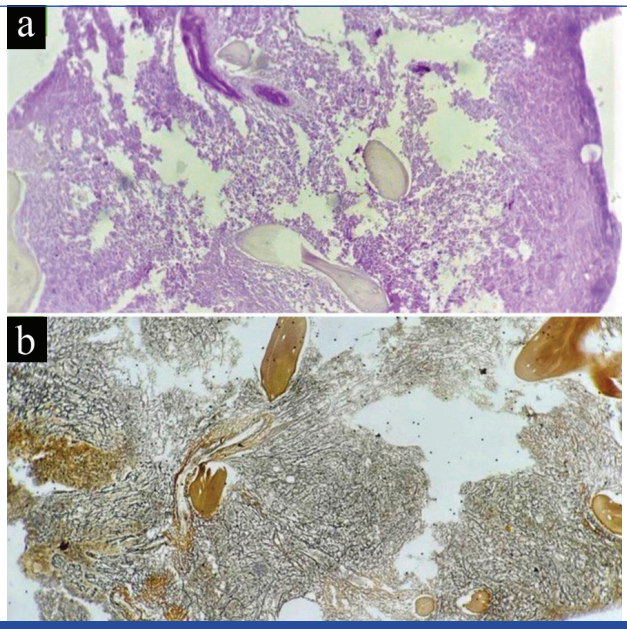


[Table/Fig-6]: Acute Leukaemia: a) BMA LG, X400; b) BMB H&E X400.

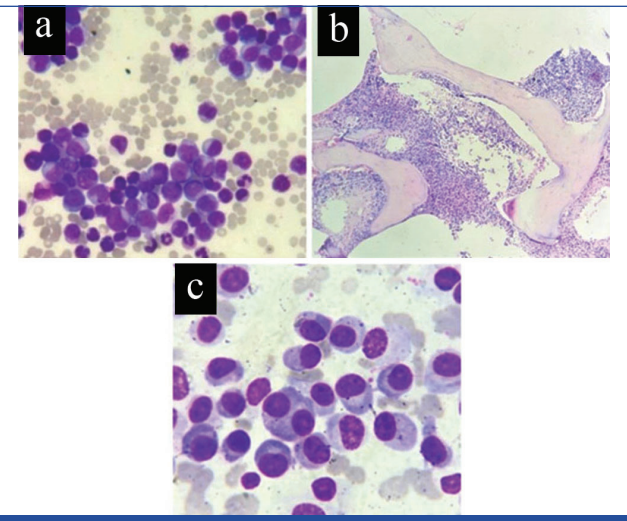
(29 cases), lymphoplasmacytosis (seven cases), and non specific myeloid reaction (four cases), BMB was not performed, especially in children <12 years of age. Hence, diagnostic accuracy was not calculated in such cases (7.7%).

Diagnosis were exclusively made by BMB in cases of myelofibrosis (six cases), granulomatous disease (three cases), and Hodgkin lymphoma (two cases), while diagnosis of hypoplastic anaemia (10 cases), acute leukaemia (six cases), marrow infiltration by Non Hodgkin lymphoma (four cases), megaloblastic anaemia (four cases), myelodysplastic syndrome (three cases), myeloproliferative disorder (three cases), plasma cell dyscrasia (two cases), and metastasis (one case) were detected mainly by BMB, with a normal and unsatisfactory marrow study on aspiration smears [Table/Fig-7-9].

The overall diagnostic accuracy of BMA cytology in diagnosing haematological and non haematological disorders was 89.96%, and the diagnostic accuracy of BMB was 96.86% with a p-value of 0.001, which was statistically significant (p-value <0.05) by the chi-square test.



[Table/Fig-7]: Myelofibrosis: a) BMB H&E, X200; b) BMB Reticulin, X200.

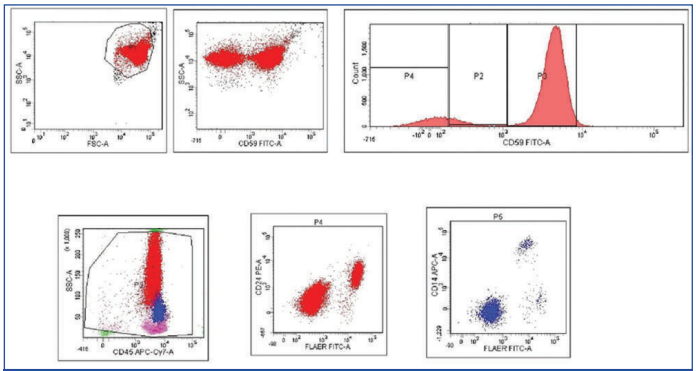


[Table/Fig-8]: a) CML in Blast crisis, BMA LG, X200; b) Lymphoma infiltration, BMB H&E, X200; c) Plasma cell dyscrasia, BMA LG, X400.

Categorisation of cases on FCM: A total of 150 cases were run on the FCM machine. A total of 128 cases were run for the Leukaemia/Lymphoma panel, out of which 118 were confirmative and 10 were non conclusive due to degenerative changes or the presence of reactive lymphocytosis. Twenty-two cases with pancytopenia on peripheral blood were run for the PNH panel, out of which 02 were positive [Table/Fig-10].

Trephine biopsy diagnosis	Bone Marrow Aspiration (BMA) findings			
	Normal marrow study	Aparticulate haemodiluted	Dry tap	Total
Normal marrow study	32	--	--	32
Megaloblastic anaemia	01	03	--	04
Hypoplastic anaemia	--	10	--	10
Acute leukaemia	--	04	02	06
Lymphoma	02	03	01	06
Myelofibrosis	--	02	04	06
Myeloproliferative disorder	--	03	--	03
Myelodysplastic syndrome	01	02	--	03
Plasma cell dyscrasia	01	01	--	02
Granulomatous disease	02	01	--	03
Metastasis	--	--	01	01
Inadequate	--	15	--	15
Total	**39**	**44**	**08**	**91**

[Table/Fig-9]: Cases diagnosed on trephine biopsy with normal and unsatisfactory aspiration study.



[Table/Fig-10]: Flow Cytometry (FCM) findings in Paroxysmal Nocturnal Haemoglobinuria (PNH).
Peripheral blood immunophenotyping SSC versus CD59 gating dot plot and histogram showed reduced/absent expression of CD 59 in RBCs; SSC Vs CD45 showed reduced/absent expression of FLAER in neutrophils and monocytes

Acute leukaemia cases were mostly categorised on BMA using special stains like PAS, MPO, and Sudan Black. However, in 18 cases, further categorisation was not done because of a lack of differentiation on morphology and inconclusive special staining. Similarly, on BMB, IHC helped in further categorisation; however, in 06 cases, still, definite characterisation was not done because of low cellularity or inconclusive IHC as antigen retrieval may be lost during routine tissue processing. FCM was helpful in confirmation, exact cell counting, and further characterisation of all 118 leukaemia/lymphoma cases [Table/Fig-11].

DISCUSSION

The BMA and BMB are important diagnostic procedures for the diagnosis of various haematological and non haematological disorders. The spectrum of haematological conditions is very wide; therefore, bone marrow examination is a useful test to reach a final diagnosis. This study was conducted to perform a comparative evaluation among BMA, BMB, and flow cytometry to determine their diagnostic utility in various diseases. Flow cytometry was used in cases of acute leukaemia, CLPD, and PNH only.

In present study, the age of the patients ranged from three months to 86 years, with a male to female ratio of 1.3:1 and a mean age of 38.4 years. The procedure was performed more frequently in adults and children under 12 years of age. The results are similar to the studies conducted by Verma S et al., and Thiyagarajan P et al., with age ranges of six months to 76 years and 8 to 90 years, respectively, and with similar male-to-female ratios in both [2,5].

S. No.	Flow Cytometry (FCM) diagnosis	Total	
1.	**Acute leukaemia panel**	**Individual**	**Cumulative**
	Acute myeloid leukaemia	**24**	**40**
	Acute promyelocytic leukaemia	**04**	
	Acute myeloid leukaemia with monocytic differentiation	**12**	
	Acute Lymphoblastic Leukaemia- B- cell type	**34**	**42**
	Acute Lymphoblastic Leukaemia- T- cell type	**08**	
	Chronic myeloid leukaemia- blast phase • Myeloid blast crisis • Lymphoid blast crisis • Mixed Blast Crisis (B-Myeloid)	**03** **03** **02**	**08**
	Degenerated/Non conclusive	**04**	**04**
2.	**CLPD panel**		
	Chronic Lymphocytic Leukaemia- B-cell type	**12**	**13**
	Chronic Lymphocytic Leukaemia- T-cell type	**01**	
	B-cell Non Hodgkin Lymphoma, possibly Mantle cell type	**02**	**15**
	B-cell Non Hodgkin Lymphoma, possibly Follicular cell type	**01**	
	B-cell Non Hodgkin Lymphoma	**12**	
	Reactive lymphocytosis/Non conclusive	**06**	**06**
3.	**PNH panel**		
	Positive	**02**	**22**
	Negative	**20**	
	Total	**150**	

[Table/Fig-11]: Categorisation of cases on Flow Cytometry (FCM).

The most common indication for bone marrow examination in present study was anaemia (31%), followed by suspected malignancy (20%) and pancytopenia (13%). A study conducted by Thiyagarajan P et al., on 153 patients also showed anaemia (33%) to be the most common indication, followed by pancytopenia (26%) and malignancy (17%) [5]. However, a study conducted by Mirzai AZ et al., on 1154 cases showed pancytopenia for evaluation to be the most common indication [6]. Another study by Bashawri LA on a total of 1813 cases showed that evaluation of acute leukaemia and staging of lymphomas were the most common indications (22.2% and 15.2%, respectively) [7]. This discrepancy can be explained by the higher incidence of unexplained anaemias among people in rural areas in present study.

In the present study, the maximum number of cases were of anaemia (35.3% and 31.7%, respectively) and leukaemia (24.7% and 25.9%, respectively), followed by a normal marrow study (7.5% and 6.2%) and megakaryocytic thrombocytopenia (4.6% each). Among anaemia cases, the majority were megaloblastic (33.8% and 32.9%, respectively) followed by hypoplastic/aplastic type (21.8% and 30.5%, respectively). Among leukaemia cases, acute leukaemia (59.4% and 61.2%, respectively) outnumbered the chronic leukaemia cases (40.6% and 38.8%, respectively) in both BMA and BMB. In acute leukaemia, ALL cases were more common than AML. In a study done by Ranabhat S et al., anaemia was the largest group (76.7%), followed by malignancy (18.9%), infection (1.9%), and miscellaneous diseases (2.5%) [8].

The study by Mahajan V et al., found the most common haematological disorder to be anaemia in 173 cases (37.6%), with megaloblastic anaemia being the most common (18.47%) [9]. Shastry SM and Kolte SS found megaloblastic anaemia to be the most common benign disorder, while acute myeloid leukaemia was the commonest haematological neoplasm [10]. Verma N et al., studied 50 cases, with the maximum number of cases being anaemia (52%), followed by leukaemia (34%), lymphoma (10%), multiple myeloma (2%), myelofibrosis (2%), leishmaniasis (2%), and idiopathic thrombocytopenic purpura (2%) [11].

In the present study, both BMA and BMB were performed in 478 out of a total of 518 cases, out of which 419 (87.6%) showed comparable results between BMA and BMB. In the remaining 59 cases (12.4%), the diagnosis could not be made on BMA due to haemodiluted bone marrow aspirate (44 cases) or dry tap (8 cases), where no comments/opinion was possible, and in cases with focal marrow involvement (7 cases), BMB was diagnostic in these cases. The results were similar to the study done by Nanda A et al., with comparable results in 88.6% and utility of BMB in 11.4% [12]. Hota R et al., also observed the positive correlation between both procedures [1]. However, the study by Vijayamohanan L et al., found that only 8.87% of cases were diagnosed by BMB alone, with an overall 68.67% concordance in findings between BMB and aspiration [13]. Vijayamohanan L et al., found micronormoblastic anaemia to be the most common benign disorder (33.72% of aspirate diagnosis and 35.50% of biopsies), followed by megaloblastic anaemia; leukaemia was the most common malignancy (15.38% of aspirates and 9.92% of biopsies) [13].

In present study, the diagnosis was exclusively made by BMB in cases of myelofibrosis, granulomatous disease, and Hodgkin's lymphoma. This highlights the importance of BMB as a key supplementary procedure in accurate diagnosis, particularly in cases of focal marrow involvement and inadequate bone marrow sampling due to extensive marrow fibrosis and hypercellularity.

Unsatisfactory bone marrow aspirates missed some cases of hypoplastic anaemia, marrow infiltration by lymphoma/leukaemia, megaloblastic anaemia, myelodysplastic syndrome, myeloproliferative disorder, and plasma cell dyscrasia. Therefore, the finding of a bloody/dry tap on BMA should never be dismissed as being due to faulty technique and always warrants a bone marrow biopsy. BMB is necessary for making a diagnosis when there is incomplete information provided by aspiration.

These findings were similar to the study by Kaur M et al., who stated that the use of biopsy avoids misinterpretation of cellularity by smears [14]. Hota R et al., also stated that the role of BMB is not only in the differentiation of MPN but also to assess the overall marrow cellularity, histo-topographic distribution of cells, morphology of megakaryocytes, as well as blasts and the degree of myelofibrosis [1]. Bearden JD et al., observed that the combined procedures of aspiration and biopsy yield higher results and are essential in patients with leukaemia and lymphoma. This is because aspiration may not be able to obtain the closely packed cells within nodules or the lymphomatous infiltrates, and the relatively normal areas may be easier to aspirate [15].

The present study observed that the diagnostic accuracy of BMB was higher (96.86%) compared to BMA (89.96%) in diagnosing various haematological and non haematological disorders. Therefore, BMB was considered the gold standard over aspiration cytology. These results were similar to the studies done by Parajuli S and Tuladhar A; Garg S and Khushnood M; and Bashir N et al., with higher diagnostic accuracy of 98.87%, 100%, and 97.3%, respectively on BMB [16-18]. However, the diagnostic accuracy of BMA was lower in the studies done by Chandra S and Chandra H (77.5%) and Aljadayeh M et al., (76.2%) [Table/Fig-12] [19,20].

In the present study, FCM was helpful in confirming and further categorising 118 cases of leukaemia/lymphoma. Present study results were similar to the study done by Hota R et al., in which seven out of 23 cases of acute leukaemia had FCM done, and in three cases, cytogenetic studies were performed. The interpretations were correlated with the BMA and BMB interpretations, showing a strong link between FCM and cytogenetic studies with BMA and BMB for giving a definitive diagnosis in different haematological malignancies. Among the 30 cases of NHL, FCM was done in four cases, which provided a confirmatory diagnosis [1].

Study	Total number of cases studied	Diagnostic accuracy of BMA	Diagnostic accuracy of BMB
Chandra S and Chandra H [19] (2011, Uttarakhand, India)	565	77.5%	99.2%
Parajuli S and Tuladhar A [16] (2014, Kathmandu, Nepal)	89	84.26%	98.87%
Aljadayeh M et al., [20] (2015, Amman-Jordan)	500	76.20%	91.80%
Garg S and Khushnood M, [17] (2017, Udaipur, India)	30	93.33%	100%
Bashir N et al., [18] (2018, Jammu and Kashmir, India)	300	96%	97.3%
Present study (2024, Haryana, India)	518	89.96%	96.86%

[Table/Fig-12]: Comparison of diagnostic accuracy of BMA and BMB in different studies [16-20].

Present study found that BMA, BMB, and FCM are complementary to each other. BMA provides excellent cytomorphological details that help in recognising abnormal haematopoietic cells or non native cells in the case of non haematological disorders. Meanwhile, BMB demonstrates the topographical arrangement of haematopoietic cells within the marrow and gives a more representative view of the cellularity of the marrow, allowing for the early recognition of marrow infiltration. On the other hand, FCM provides a definitive idea about the origin and type of the cell in different haematological malignancies and adds a strong diagnostic confirmation to the BMA and BMB interpretations.

Limitation(s)

Present study did not evaluate touch imprint smears, which may increase the diagnostic accuracy, and there were small numbers of cases in each subgroup.

CONCLUSION(S)

The BMA provided a rapid diagnosis in a variety of disorders; however, BMB is a complementary and necessary procedure in providing an accurate diagnosis. Despite its disadvantages, it is recommended for routine performance of a sequential aspiration followed by a biopsy in all cases wherever possible to facilitate a proper diagnostic work-up. In cases with suspected leukaemia/lymphoma and PNH, FCM is a definite diagnostic modality. Further studies with a greater number of cases and simultaneous cytogenetic evaluation will provide definitive evidence of genetic alterations in various haematological malignancies, which will help in understanding the prognostic index, patient's survival, and targeted therapy.

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