Original Article

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Pathology Section

Diagnostic Accuracy of Peripheral Blood Film Examination in Comparison to Bone Marrow Aspiration in Various Haematological Disorders: A Cross-sectional Study

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ABSTRACT

Introduction: Bone marrow aspiration is a routine diagnostic procedure performed in hospitals for the diagnosis and management of haematological disorders. It involves cytomorphological assessment of all blood cells through bone marrow aspiration and Peripheral Blood Film (PBF) examination.

Aim: To analyse the diagnostic accuracy of PBF in comparison to bone marrow aspiration for diagnosing various haematological diseases in southern Rajasthan, India.

Materials and Methods: This cross-sectional study was conducted at the Department of Pathology, Rabindra Nath Tagore (RNT) Medical College, Udaipur Rajasthan, India, from January 2016 to June 2022. The study included microscopic findings of 638 bone marrow cases along with corresponding PBF and complete blood count findings. The results were entered into an MS Excel sheet and evaluated using Statistical Package for the Social Sciences (SPSS) 22.00 software. Chi-square test was used to calculate p-values.

Results: The study included a total of 638 bone marrow aspiration cases, with 368 cases (57.68%) being male and 270 cases (42.32%) being female. The mean age was 30.8 years (4 months to 87 years). The most common bone marrow diagnosis was Erythroid Hyperplasia, with 187 cases (29.31%) (including micronormoblastic, megalonormoblastic, normoblastic, and nutritional), followed by Chronic Myeloid Leukaemia (CML) with 154 cases (24.14%), and acute leukaemia with 152 cases (23.82%). Among the benign diagnoses, micronormoblastic erythroid hyperplasia was the most common with 68 cases (27.09%). Among the malignant diagnoses, CML was the most common with 154 cases (39.79%). The sensitivity of PBF diagnosis was 100% for Chronic Lymphocytic Leukaemia (CLL), CML, and Prolymphocytic Leukaemia (PLL), and 82.24% for acute leukaemia; 45.5% for lymphoma infiltration. The specificity was 100% for all cases. The accuracy was 95.75% for acute leukaemia, 99.06% for lymphoma infiltration, and 100% for CML, CLL, PLL, and myeloproliferative disorder-eosinophilia. The p-value was <0.001 for Acute Leukaemia, CML, CLL, PLL, myeloproliferative disorder-eosinophilia, and <0.002 for lymphoma infiltration. The overall sensitivity, specificity, accuracy, and p-value were 84.5%, 100%, 90.6%, and <0.001, respectively.

Conclusion: While bone marrow examination is crucial for confirming the diagnosis of haematological disorders, PBF examination alone can be used for phasing and confirming the diagnosis of CML and CLL.

Keywords: Anaemia, Bone marrow examination, Haematological diseases, Leukaemia

INTRODUCTION

Bone marrow aspiration is the most frequent and safe invasive procedure routinely performed in hospitals to evaluate various neoplastic and non neoplastic haematological disorders [1]. It is done after a proper assessment of medical history, physical examination, blood count analysis, and examination of peripheral smear morphology [2]. It is very helpful in confirming clinically suspected diseases and providing previously unsuspected diagnoses [3].

The concomitant examination of bone marrow aspiration and PBF examination is conclusive as it provides information regarding the morphology and maturation of all lineages of blood cells [4]. It also helps in finding out prognosis based on staging and assessing disease staging before and after chemotherapy [5]. Other analyses such as flow cytometry, microbiological tests, cytogenetics, molecular genetics, and immunophenotyping can also be performed on bone marrow aspirate [2,6]. In various haematological diseases, there is a disordered numerical and spatial relationship among bone marrow cells and changes in cell appearance. The spectrum of haematological disorders diagnosed with bone marrow aspiration examination ranges from common disorders like nutritional deficiency anaemia to life-threatening conditions like leukaemia [4].

Bone marrow aspiration can be performed in severe thrombocytopenia with a minimum risk of bleeding [1]. However, many contraindications also exist for this procedure such as Haemorrhagic disorders, disseminated intravascular coagulation, anticoagulant drugs; sampling site skin infection, and bone disorders such as osteomyelitis or osteogenesis imperfecta [7]. This study analysed the diagnostic accuracy of PBF in comparison to bone marrow aspiration for diagnosing various haematological diseases and to study the spectrum of various haematological diseases in different age groups in southern Rajasthan, India. The study helps to establish the use of PBF for diagnosing various haematological malignancies in resource-poor settings.

MATERIALS AND METHODS

The present study was a cross-sectional study conducted from January 2016 to June 2022 in Department of Pathology, Rabindranath Tagore Medical College and Associated Hospitals in Udaipur, Rajasthan, India. A total of 638 bone marrow aspiration cases received in the haematology laboratory during that duration were included in the study. As it was a laboratory-based cross-sectional study, there was less than minimal risk to the patients, and the data related to identity were kept highly confidential. Clinical

data, including the mode of onset, history of previous illness and treatment, bone pains, fever, bleeding, hepatosplenomegaly, lymphadenopathy, Complete Blood Cell (CBC) count, and PBF, were recorded.

Inclusion criteria: All bone marrow aspiration smears with available CBC count and PBF were included in the study.

Exclusion criteria: All unsatisfactory diluted bone marrow aspiration smears and smears with non availability of clinical history and relevant data were excluded from the study.

Preparation of a peripheral blood smear: About 2 to 3 mL of blood was collected in an Ethylenediaminetetraacetic Acid (EDTA) anticoagulated vial from the antecubital vein. A drop of blood was taken and placed on a clean glass slide, 1 cm from the end. A smear was made using the wedge method, creating tongue-shaped smears of about 2.5 to 3.5 cm in length. At least two smears were prepared. The smears were air-dried, fixed in methanol, and stained with an in-house made Giemsa stain. Bone marrow aspiration was done using Salah's bone marrow puncture needle. The preferred site was the posterior superior iliac spine. Smears were made, air-dried, fixed in methanol, and stained with Giemsa stain. PBF findings were compared with bone marrow aspiration results, as bone marrow biopsy was available for very few cases.

Microscopic examination of smears: PBF examination included a low-power scan to check for the presence of hemoparasites, a 40x examination to assess Red Blood Cell (RBC) and White Blood Cell (WBC) morphology and count, and an oil immersion examination to evaluate blast cell morphology and platelet count. Bone marrow smear examination involved a low-power scan to assess sample adequacy, as well as high power and oil immersion lens examinations to determine cellular morphology and distribution. The smears were assessed for cellularity, differentiation, and maturation of the erythroid, myeloid, and megakaryocytic lineages, as well as the presence of plasma cells, lymphocytes, parasites/abnormal cells/granulomas/storage cells. The myeloid: erythroid ratio was calculated, and malignant haematological disorders were categorised using the 5th edition of the WHO classification of haematolymphoid tumours [8,9].

STATISTICAL ANALYSIS

The collected data was entered into an Excel sheet and analysed using SPSS version 22.00 software. The mean, specificity, sensitivity, p-value using the chi-square test, and accuracy were calculated.

Senstivity=(True Positive/True Positive +False Negative) *100

Specificity=(True Negative/False Positive+True Negative) *100

Accuracy=(True positive+True Negative/True Positive+True Negative+False Positive+False Negative) *100

RESULTS

Out of the 638 cases included in the study, 368 (57.68%) were male and 270 (42.32%) were female [Table/Fig-1]. The mean age was 30.8 years (4 months to 87 years).

As depicted in [Table/Fig-2,3], hypoplastic anaemia (18 cases), anaemia's including megaloblastic (46 cases), micronormoblastic (37 cases), and nutritional anaemias (33 cases), as well as ITP (11 cases) with acute leukaemia (123 cases), showed the highest number of cases in the 0-20 years age group. The number of cases of CML was 78, and plasma cell dyscrasias had 11 cases, with the highest occurrence observed in the 41-60 years age group. CLL was more common in the 61-80 years age group, with 26 cases reported.

The highest number of diagnoses based on PBF results were CML with 154 cases (24.1%), followed by pancytopenia with 147 cases (23.04%), acute leukaemia with 125 cases (19.6%), and exclusive anaemia with 94 cases (14.73%), including dimorphic, microcytic, macrocytic, and normocytic anaemias [Table/Fig-1].

PBF diagnosis	Female	Male	Grand total	Percentage
Acute leukaemia	53	72	125	19.6
CML	69	85	154	24.1
CLL	11	30	41	6.4
PLL	-	1	1	0.15
Suspicious of subleukaemic leukaemia	2	1	3	0.5
Atypical lymphoid cells	1	4	5	0.8
Myeloproliferative disorder	1	-	1	0.15
Microcytic hypochromic anaemia	19	18	37	5.8
Normocytic normochromic anaemia	11	24	35	5.5
Macrocytic normochromic anaemia	3	5	8	1.3
Dimorphic anaemia	7	7	14	2.2
Neutrophilia	5	3	8	1.2
Lymphocytosis	1	1	2	0.3
Eosinophilia	-	2	2	0.3
Pancytopenia	61	80	141	22.1
Pancytopenia LD bodies	-	1	1	0.15
Pancytopenia malaria	2	3	5	0.8
Bicytopenia	14	17	31	4.9
Thrombocytopenia	8	2	10	1.6
Leucopenia	-	1	1	0.15
Normal	2	11	13	2.0
Grand Total	270	368	638	100.0

[Table/Fig-1]: Sex distribution of various PBF diagnoses.
CML: Chronic myeloid leukaemia; CLL: Chronic lymphocytic leukaemia; PLL: Prolymphocytic leukaemia

Regarding bone marrow diagnoses, the highest number of cases were erythroid hyperplasia with 187 cases (29.31%), including micronormoblastic, megalonormoblastic, normoblastic, and nutritional types. This was followed by CML with 154 cases (24.14%), and acute leukaemia with 152 cases (23.82%), as presented in [Table/Fig-2,3].

Among the benign bone marrow diagnoses, the highest number of cases were micronormoblastic erythroid hyperplasia with 68 cases (27.09%), followed by megalonormoblastic erythroid hyperplasia with 59 cases (23.51%), and nutritional anaemia with 44 cases (17.53%), as shown in [Table/Fig-4].

In malignant bone marrow diagnoses, the highest number of cases were CML with 154 cases (39.79%), followed by acute leukaemia with 152 cases (39.27%), CLL with 41 cases (10.6%), and plasma cell dyscrasia with 16 cases (4.14%), as shown in [Table/Fig-5-7]. The sensitivity, specificity, accuracy, and p-value of PBF examination results for certain malignant haematological disorders were calculated, considering bone marrow aspiration smear examination as the gold standard.

The sensitivity of PBF examination was 100% for CLL, CML, and PLL; 82.24% for acute leukaemia; 45.5% for lymphoma infiltration, and 0% for Immune Thrombocytopenic Purpura (ITP), plasma cell dyscrasia, and metastasis. The specificity was 100% for all cases, as shown in [Table/Fig-8].

The accuracy was 95.75% for acute leukaemia, 99.06% for lymphoma infiltration, and 100% for CML, CLL, PLL, and myeloproliferative disorder-eosinophilia. The p-value was <0.001 for acute leukaemia, CML, CLL, PLL, and myeloproliferative disorder-eosinophilia, and <0.002 for lymphoma infiltration, indicating statistical significance. The overall sensitivity, specificity, accuracy, and p-value of PBF for diagnosing malignant cases were 84.5%, 100%, 90.6%, and <0.001, respectively.

S. No.	Benign bone marrow diagnosis	Sex		Age groups (in years)					Grand total	Percentage out of benign cases	Percentage out of total cases
		M	F	0-20	21-40	41-60	61-80	81-100			
1.	Micronormoblastic erythroid hyperplasia	31	37	37	11	9	10	1	68	27.09	10.66
2.	Normoblastic erythroid hyperplasia	9	7	4	7	3	2		16	6.37	2.51
3.	Megalonormoblastic erythroid hyperplasia	37	22	46	5	5	3		59	23.51	9.25
4.	Nutritional anaemia	25	19	33	5	3	3		44	17.53	6.90
5.	Hypoplastic anaemia	11	7	18					18	7.17	2.82
6.	ITP	5	9	11	3				14	5.58	2.19
7.	Malaria	3	2	5					5	1.99	0.78
8.	Leishmaniasis	1	0		1				1	0.4	0.16
9.	Leukemoid reaction	2	4	2	1	1	2		6	2.39	0.94
10.	Amegakaryocytic thrombocytopenia	1	0	1					1	0.4	0.16
11.	Granuloma	1	1	1	1				2	0.8	0.31
12.	Normal marrow study	15	2	6	3	6	1	1	17	6.77	2.66
13.	Total	141	110	164	37	27	21	2	251	100	39.34
Percentage				65.34	14.74	10.76	8.37	0.79		100	

[Table/Fig-2]: Age and sex distribution of various benign bone marrow diagnosis.
ITP: Immune thrombocytopenic purpura

S. No.	Malignant bone marrow diagnosis	Sex		Age groups (in years)					Grand total	Percentage out of malignant cases	Percentage out of total cases
		M	F	0-20	21-40	41-60	61-80	81-100			
1.	Acute leukaemia	86	66	123	4	11	14		152	39.27	23.82
2.	CML	85	69	7	45	78	24		154	39.79	24.14
3.	CLL	30	11			13	26	2	41	10.6	6.42
4.	PLL	1	0				1		1	0.26	0.16
5.	Plasma cell dyscrasia	10	6			11	5		16	4.14	2.51
6.	Lymphoma infiltration	8	3	2	1	6	2		11	2.84	1.72
7.	Metastatic lesion	5	2	1	1	2	3		7	1.81	1.10
8.	Myeloproliferative disorder- Eosinophilia	0	1			1			1	0.26	0.16
9.	MDS	2	2			1	2	1	4	1.03	0.63
	Total	227	160	133	51	123	77	3	387	100	60.66
	Percentage			34.37	13.18	31.78	19.90	0.77	100.00		

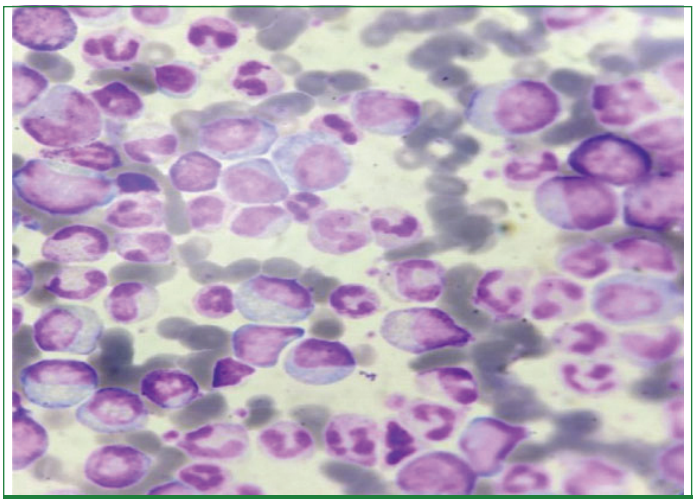
[Table/Fig-3]: Age distribution of various malignant bone marrow diagnosis.
CML: Chronic myeloid leukaemia; CLL: Chronic lymphocytic leukaemia; PLL: Prolymphocytic leukaemia; MDS: Myelodysplastic syndrome

S. No.	BM diagnosis	No. of cases	PBF diagnosis	No. of cases
1.	Micronormoblastic erythroid hyperplasia	68	Bicytopenia	8
			Dimorphic anaemia	1
			Microcytic hypochromic anaemia	29
			Normocytic normochromic anaemia	1
			Pancytopenia	29
2.	Normoblastic erythroid hyperplasia	16	Bicytopenia	1
			Normocytic normochromic anaemia	11
			Pancytopenia	4
3.	Megalonormoblastic erythroid hyperplasia	59	Bicytopenia	6
			Dimorphic anaemia	1
			Macrocytic normochromic anaemia	8
			Pancytopenia	44
4.	Nutritional anaemia	44	Bicytopenia	8
			Dimorphic anaemia	12
			Microcytic hypochromic anaemia	1
			Normocytic normochromic anaemia	1
			Pancytopenia	22
5.	Hypoplastic anaemia	18	Pancytopenia	17
			Leucopenia	1
6.	ITP	14	Bicytopenia	4
			Pancytopenia	1
			Thrombocytopenia	9
7.	Amegakaryocytic thrombocytopenia	1	Thrombocytopenia	1
8.	Malaria	5	Pancytopenia with malarial parasite	5
9.	Leishmaniasis	1	Pancytopenia	1
10.	Granuloma	2	Lymphocytosis	2
11.	Leukemoid reaction	6	Neutrophilia	6
12.	Normal marrow study	17	Eosinophilia	2
			Microcytic hypochromic anaemia	2
			Neutrophilia	1
			Normal	6
			Normocytic normochromic anaemia	5
			Pancytopenia	1
Total number of non-malignant cases		251		251

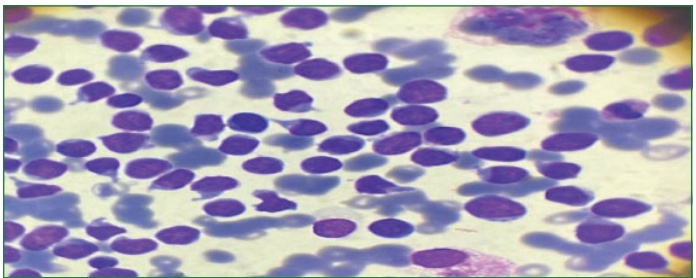
[Table/Fig-4]: PBF findings of non malignant bone marrow diagnosis.

S. No.	Bone marrow diagnosis	No. of case	PBF diagnosis	No. of case
1.	Acute leukaemia	152	Acute leukaemia	125
			Bicytopenia	4
			Normocytic normochromic anaemia	1
			Pancytopenia	19
			Suspicious of subleukaemic leukaemia	3
2.	CML	154	CML	154
3.	CLL	41	CLL	41
4.	Lymphoma infiltration	11	Atypical lymphoid cell	5
			Normal PBF	6
5.	Plasma cell dyscrasia	16	Microcytic hypochromic anaemia	3
			Normocytic normochromic anaemia	12
			Pancytopenia	1
6.	PLL	1	PLL	1
7.	Myeloproliferative disorder- eosinophilia	1	Myeloproliferative disorder-eosinophilia	1
8.	Metastatic lesion	7	Microcytic hypochromic anaemia	2
			Neutrophilia	1
			Normal	1
			Normocytic normochromic anaemia	2
			Pancytopenia	1
9.	MDS	4	Normocytic normochromic anaemia	2
			Pancytopenia	2
Total malignant diagnosis		387		387

[Table/Fig-5]: PBF findings of malignant bone marrow diagnosis.



[Table/Fig-6]: Microphotograph of PBF examination (100x) of Chronic Myeloid Leukaemia (CML) stained in Giemsa stain.



[Table/Fig-7]: Microphotograph of PBF examination (100 x) of acute leukaemia stained in Giemsa.

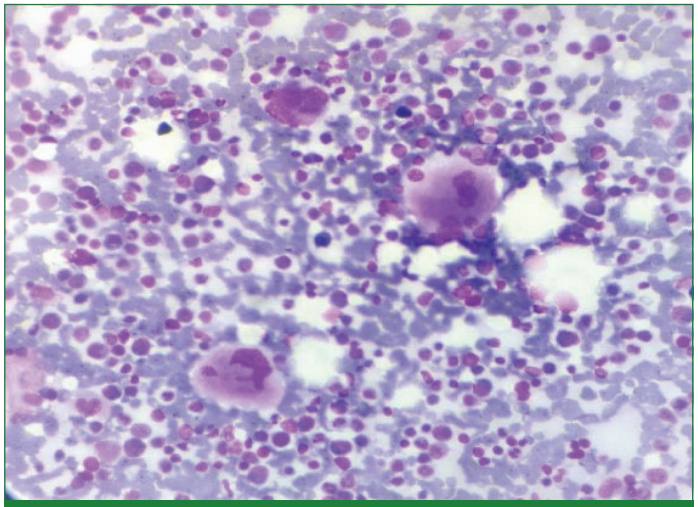
Using Giemsa stain, the cytomorphological features of ITP and plasma cell dyscrasia were studied, as shown in [Table/Fig-9,10].

DISCUSSION

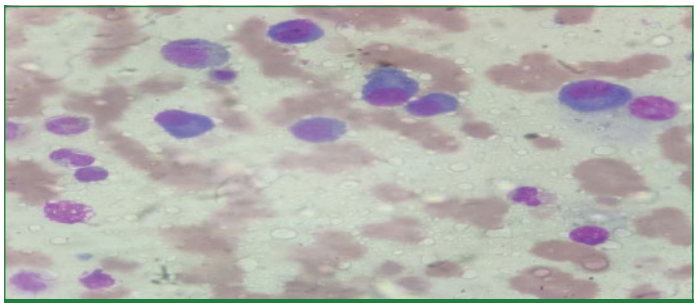
In this study, there were 251 cases (39.34%) of benign haematological diagnoses, while 387 cases (60.66%) were malignant diagnoses. The

S. No.	Diagnosis	PBF	BM	Sensitivity (%)	Specificity (%)	Accuracy (%)	p-value
1.	Acute leukaemia	125	152	82.24	100	95.75	<0.001
2.	CML	154	154	100	100	100	<0.001
3.	CLL	41	41	100	100	100	<0.001
4.	PLL	1	1	100	100	100	<0.001
5.	Plasma cell dyscrasia	0	16	0	100	-	-
6.	Lymphoma infiltration	5	11	45.5	100	99.06	<0.002
7.	Metastatic lesion	0	7	0	100	-	-
8.	Myeloproliferative disorder-eosinophilia	1	1	100	100	100	<0.001
9.	MDS	0	4	0	100	-	-
	Total case	327	387	84.5	100	90.6	<0.001

[Table/Fig-8]: Diagnostic accuracy of PBF examination in comparison to BM aspiration examination in malignant cases.



[Table/Fig-9]: Microphotograph of BM examination (10x) of Immune Thrombocytopenic Purpura (ITP) stained in Giemsa stain.



[Table/Fig-10]: Microphotograph of BM examination (100x) of plasma cell dyscrasia stained in Giemsa stain.

number of malignant cases was higher compared to other similar studies such as Gandapur AS et al., (27.8%) and Bagale P and Shingade M (22%) because bone marrow aspiration was preferentially performed on suspicious malignant cases [10,11].

This study showed a male preponderance with a male-to-female ratio of 4:3, which was comparable to studies conducted by Raina JS et al., (3:2), Manzoor F et al., (1.6:1), and Biradar MP et al., (2:1) [6,12,13]. Male preponderance was in accordance with clinical-epidemiological studies, which have stated that men are more prone to haematological disorders.

According to this study, the age of cases ranged from 4 months to 87 years, which was comparable to the studies conducted by Raina JS et al., (1 year to 90 years), Das S et al., (4 years to 87 years), Gayathri BN and Rao KS (2 to 80 years) (2011), and Vaidya S (4 to 75 years) (2015) [6,14-16]. The age group with the highest number undergoing bone marrow aspiration was 0-20 years

(297 cases, 46.55%), whereas Shastri SM and Kolte SS had 28 cases (25.45%) in the 21-30 years age group and 22 cases (20%) in the 11-20 years age group [17]. As depicted in [Table/Fig-2], cases of hypoplastic anaemia, anaemias including megaloblastic, micronormoblastic, and nutritional anaemias, and ITP with acute leukaemia showed the highest number of cases in the 0-20 years age group.

CML and plasma cell dyscrasias were most common in the 41-60 years age group, whereas CLL was common in the 60-80 years age group. Erythroid hyperplasia was the most common diagnosis in bone marrow aspiration, accounting for a total of 29.31% in present study. Similar results were seen in studies by Pudasaini S et al., (21%), Khodke K et al., (14%), and Jha A et al., (19.6%) [1,18,19].

Micronormoblastic erythroid hyperplasia was observed in 68 cases (10.66%) on bone marrow aspiration, compared to a study by Al-Ghazaly J et al., (10.4%). It had the highest incidence in the 0-20 years age group and was associated with malnutrition, parasitic infestation, gastrointestinal haemorrhage, and heavy menstrual bleeding [20]. Microcytic anaemia and pancytopenia were the most common concomitant findings on PBF examination.

Megalonormoblastic erythroid hyperplasia was seen in 59 cases (9.25%), compared to other studies done by Pudasaini S et al., and Khodke K et al., Al-Ghazaly J et al., Ahmed SQ et al., which showed (12.3%), (6.5%), (9.1%), and (11.9%) of their cases, respectively [1,18,20,21].

Pancytopenia was the most common concomitant finding on PBF examination. Megaloblastic anaemia may be associated with a vegetarian diet, chronic diarrhoea, malabsorption, and malnutrition due to a low socio-economic status. It can also be caused by hereditary haemolytic anaemias in aplastic crises, pernicious anaemia, and tropical splenomegaly syndrome caused by recurrent malaria infestation leading to folic acid deficiency. Most patients presented with pancytopenia, which necessitated bone marrow aspiration for diagnosis. Concomitant vitamin B12 and folic acid assays were not performed.

Nutritional deficiency, comprising megalonormoblastic and micronormoblastic erythroid hyperplasia, was seen in 44 cases (6.90%), similar to the study by Balasubramanian M and Sangoi NN (13.3%) [4]. Pancytopenia was the most common finding associated with this condition on PBF examination. It could be attributed to low socioe-conomic status and a vegetarian diet. Hypoplastic anaemia was seen in 18 cases (2.82%), comparable to studies by Pudasaini S et al., (5.3%), Gandapur AS et al., (1.75%), and Bagale P and Shingade M (5.92%) [1,10,11]. In all cases of hypoplastic anaemia, the marrow was hypocellular and all three lineages of cells were suppressed. BMA findings were correlated with PBF, which also showed pancytopenia in these cases [Table/Fig-5]. However, bone marrow biopsy was not performed in these cases.

In this study, acute leukaemia comprised 152 cases (23.82%), similar to the study by Balasubramanian M and Sangoi NN (27.3%) [4]. Various factors such as differences in methodology, geographic distribution, genetic disturbances, preferential aspiration of suspicious leukaemia cases, and the period of observation may cause variation in the incidence of disorders causing acute leukaemia. The predominant reason for not giving a conclusive diagnosis of acute leukaemia on PBF examination in present cases was pancytopenia, bicytopenia, and subleukaemic leukaemia in decreasing order. Both PBF and bone marrow aspiration were complementary in all other cases of leukaemia diagnosed in this study.

According to this study, CML comprised the highest number of malignant diagnoses with 154 cases (24.14%), which was higher compared to other similar studies. This could be attributed to differences in patients' lifestyle and economic status, as well as the preferential availability of treatment for CML in our center compared to other leukaemias. The high frequency of leukaemia, especially in rural areas and less in urban areas, may be due to the use of pesticides in agriculture without regulations and protective measures.

In the present study, CLL comprised 41 cases (6.42%), which was similar to the study by Al-Ghazaly J et al., (5.7%) and Nasher ST et al., (3.7%) [22,20]. Plasma cell dyscrasia comprised 16 cases (2.51%), similar to the study by Pudasaini S et al., (3.5%) [1] and Jha A et al., (0.94% to 4.1%) [19], and Bagale P and Shingade M (2.22%) [11]. Both CLL and plasma cell dyscrasia were mostly seen in the older age group. Most CLL cases were diagnosed incidentally, while plasma cell dyscrasia cases presented with pathological fractures. CLL and plasma cell dyscrasia were more commonly seen in males. Myelodysplastic Syndrome (MDS) comprised four cases (0.63%), similar to Bagale P and Shingade M (0.6%) [11] and Pudasaini S et al., (3.5%) [1]. The diagnosis of MDS was given after excluding all other secondary causes of dysplasia. ITP was diagnosed in 14 cases (2.19%), similar to the study by Khodke K et al., (5%) [18]. The present study showed the maximum number of ITP cases in the 0-20 years age group, mostly in children, associated with viral infections and autoimmunity.

Infective pathology was seen in six cases, out of which five cases were diagnosed with Malarial infection and one case was diagnosed with Leishmaniasis. This study showed 0.78% of malaria cases, similar to Santra G and Das BK (1.8%) [23]. All the cases were documented during the summer to rainy season, corresponding to the prominent mosquito breeding season. Only one case (0.16%) of Leishmaniasis was seen, similar to Bagale P and Shingade M (0.25%) [11]. The patient was a migrant laborer from Nepal. Granuloma was seen in two cases (0.31% cases), similar to Das S et al., (1.16%) [14]. Both patients had a history of tuberculosis.

Lymphoma infiltration was seen in 11 cases (1.72%), similar to Dogan A et al., (2%) [2]. Peripheral blood involvement was seen in five cases (45.4%) out of the 11 cases with marrow involvement, similar to the studies by Goel N et al., and Sovani V et al., [7,24]. On follow-up, all the cases were found to be non Hodgkin's lymphoma. Metastasis was seen in seven cases (1.09%), similar to Dogan A et al., (1.8%) [2]. There were 17 cases showing normal marrow findings, which were mostly associated with normal PBF findings (six cases). These cases were suspected cases of multiple myeloma and metastasis. Other associated cases were unexplained normocytic normochromic anaemia (five cases), eosinophilia (two cases), microcytic hypochromic anaemia (two cases), and one case each of neutrophilia and pancytopenia.

The diseases diagnosed on bone marrow aspiration in cases where PBF was inconclusive were metastatic cancers, hypoplastic anaemias, lymphoma infiltration, plasma cell dyscrasia, ITP, and granuloma. Hence, for these diagnoses, bone marrow aspiration is essential [Table/Fig-5,6]. PBF examination was conclusive and showed 100% sensitivity for the diagnosis of CLL, PLL, and CML, whereas the specificity of PBF examination was 100% for all diagnoses [Table/Fig-4]. The overall sensitivity of 84.5% and accuracy of 90.6% were due to the inability of PBF examination to diagnose all cases of plasma cell dyscrasia, metastasis, MDS, and a few cases of lymphoma infiltration and leukaemias. The sensitivity of 100% ensures that PBF can exclude all cases not having haematological malignancy. The p-value of <0.001 is statistically significant. The calculated accuracy, sensitivity, specificity, and p-value show that PBF examination is a useful procedure to detect CML, CLL, PLL, and myeloproliferative disorder, but it cannot be a substitute for examination of the marrow by bone marrow aspiration and core biopsy in all other cases. The utility of PBF examination is supported by its earlier and easier availability, although the combination of PBF and bone marrow examination is best to detect all the true positives and limit false-negative diagnoses.

Limitation(s)

Bone marrow biopsy was done for only a few cases as bone marrow aspiration provides a sample for both cytomorphology and ancillary

testing (such as flow cytometry) and gives an early diagnosis within days compared to biopsy, whose results take weeks to assess.

CONCLUSION(S)

Marrow aspirate is a simple, rapid method primarily utilised for cytological assessment. It is a decisive step for the confirmatory diagnosis of haematological disorders. Bone marrow examination gives confirmatory pathological diagnosis for unexplained cytopenias and leukaemia. It also gives the type of erythroid reaction to anaemia that cannot be determined from the peripheral blood smear alone. From present study, it can be concluded that for the phasing and confirmatory diagnosis of CML and CLL, PBF examination alone was found to be sufficient.

REFERENCES

[1] Pudasaini S, Prasad KBR, Rauniyar SK, Shrestha R, Gautam K, Pathak R, et al. Interpretation of bone marrow aspiration in hematological disorder. Journal of Pathology of Nepal. 2012;2:309-12.
[2] Dogan A, Demircioglu S. Diagnostic importance of bone marrow aspiration evaluation: A single-center study. Pak J Med Sci. 2022;38(4):811-15.
[3] Parajuli S, Tuladhar A. Correlation of bone marrow aspiration and biopsy findings in diagnosing hematological disorders- A study of 89 cases. Journal of Pathology of Nepal. 2014;4:534-38.
[4] Balasubramanian M, Sangoi NN. Utility of bone marrow examination in pediatricage group: Experience of a tertiary healthcare centre in India. Cureus. 2022;14(7):01-10.
[5] Joshi S, Wader J, Kanethkar S. Bone marrow aspiration and bone marrow biopsy in hematological disorders. Biomed and Pharmacol J. 2020;13(2):799-804.
[6] Raina JS, Kundal R, Puri P, Puri A, Kumar K, Attri HK. Correlation between different blood investigations-peripheral blood film and bone marrow findings in cases of pancytopenia. IJRR. 2020;7(1):47-53.
[7] Goel N, Kaur M, Bal MS. Analysis of bone marrow and peripheral blood film findings in sixty diagnosed cases of lymphoma. IP Archives of Cytology and Histopathology Research. 2020;5(1):47-57.
[8] Alaggio R, Amador C, Anagnostopoulos I, Attygalle AD, Araujo IBO, Berti E, et al. The 5th edition of the World Health Organization Classification of haematolymphoid tumours: Lymphoid neoplasms. Leukaemia. 2022;36(7):1720-48. Doi: 10.1038/s41375-022-01620-2. Epub 2022 Jun 22. PMID: 35732829; PMCID: PMC9214472.
[9] Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the World Health Organization classification of haematolymphoid tumours: myeloid and histiocytic/dendritic neoplasms. Leukaemia. 2022;36(7):1703-19. https://doi.org/10.1038/s41375-022-01613-1.
[10] Gandapur AS, Nadeem S, Riaz M, Mannan M. Diagnostic importance of bone marrow examination in haematological malignant and non-malignant disorders. J Ayub Med Coll Abbottabad. 2015;27(3):692-94.
[11] Bagale P, Shingade M. Spectrum of haematological disorders on bone marrow aspiration and biopsy: A retrospective study of cases. IJAR. 2018;8(11):58-59.
[12] Manzoor F, Karandikar MN, Nimbargi RC. Pancytopenia: A clinico-hematological study. Medical Journal of Dr. D.Y. Patil University. 2014;7(1):25-28.
[13] Biradar MP, Biradar P, Sharada MS. A study of bone marrow examination in cases of pancytopenia. Journal of Krishna Institute of Medical Sciences University. 2016;5(4):117-21.
[14] Das S, Kerketta VM, Baruah PK. Role of bone marrow aspiration and marrow biopsy in haematological disorders. IJAR. 2022;12(7):47-48,
[15] Gayathri BN, Rao KS. Pancytopenia: A clinico hematological study. J Lab Physicians. 2011;3(1):15-20.
[16] Vaidya S. Evaluation of bone marrow in cases of pancytopenia in a tertiary care hospital. Journal of Pathology of Nepal. 2015;5:691-95.
[17] Shastri SM, Kolte SS. Spectrum of hematological disorders observed in one hundred and ten consecutive bone marrow aspirations and biopsies. Med J Dr. D.Y. Patil Univ. 2012;5(2):118-21.
[18] Khodke K, Marwah S, Buxi G, Yadav RB, Chaturvedi NK. Bone marrow examination in cases of pancytopenia. JIACM. 2001;2:55-59.
[19] Jha A, Sayami G, Adhikari RC, Panta D, Jha R. Bone marrow examination in cases of pancytopenia. J Nepal Med Assoc. 2008;47(169):12-17.
[20] Al-Ghazaly J, Al-Selwi AH, Abdullah M, Al-Jahafi AK, Al-Dubai W, Al-Hashdi A. Pattern of haematological diseases diagnosed by bone marrow examination in Yemen: A developing country experience. Clin Lab Haematol. 2006;28(6):376-81.
[21] Ahmed SQ, Khan OU, Zafar N. Utilization of bone marrow examination in a secondary care hospital. JRMC. 2011;15:40-41.
[22] Nasher ST, Alyousufy F, Alkubati K, Halimy SA, Athwary RA. Bone marrow profile in hematological disorders in Yemeni patients. IJAR. 2021;11(9):61-65.
[23] Santra G, Das BK. A cross-sectional study of the clinical profile and aetiological spectrum of pancytopenia in a tertiary care centre. Singapore Med J. 2010;51(10):806-12.
[24] Sovani V, Harvey C, Haynes AP, McMillan AK, Clark DM, O'Connor SR. Bone marrow trephine biopsy involvement by lymphoma: Review of histopathological features in 511 specimens and correlation with diagnostic biopsy, aspirate and peripheral blood findings. J Clin Pathol. 2014;67(5):389-95.

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