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Correlation between Different Blood Investigations - Peripheral Blood Film and Bone Marrow Findings in Cases of Pancytopenia

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ABSTRACT

Introduction: Pancytopenia is the simultaneous presence of anaemia, leucopenia and thrombocytopenia. Varieties of hematological and non-hematological disorders may affect bone marrow either primarily or secondarily, resulting in the manifestation of pancytopenia. The present study was done to correlate the peripheral blood film and bone marrow findings in cases of pancytopenia.
Material and Method: The present study was conducted among 60 patients in the Pathology department of Government Medical College, and Rajindra Hospital, Patiala, under Professor & Head of Pathology Dr. Arun Puri. Bone marrow aspiration was done by using Salah's bone marrow puncture needle. The smears were assessed for cellularity, differentiation and maturation of erythroid, myeloid and megakaryocytic lineage, M:E ratio, Plasma cells, Lymphocytes and parasites/ abnormal cells/granulomas/storage cells.
Results: The mean range of Hb level observed was 4.94±1.72. Out of 60 patients, the predominant RBC picture was dimorphic in 53patients (88.30%) followed by normocytic in 6 patients (10%) and microcytic in1 patient (1.70%). Total 46 (100%) patients of megaloblastic anemia had macrocytes on peripheral blood film and 35 patients (76%) had hypersegmented neutrophils correlated to bone marrow aspiration which showed hypercellular marrow with erythroid hyperplasia in total 46 patients of megaloblastic anemia
Conclusion: The results of the present study showed that there is a significant correlation between bone marrow aspiration findings and peripheral blood film findings. Both the investigations are complementary to each other in evaluating the patient.
Keywords: Hb, Bone Marrow, Pancytopenia, Anemia

INTRODUCTION

Pancytopenia is reduction in all the three major cellular elements of blood; hence it is the simultaneous presence of anaemia, leucopenia and thrombocytopenia. It is not a disease entity but a triad of findings that may result from various disease processes, primarily or secondarily involving the bone marrow. [1] The complete hematological work up with good clinical correlation is of utmost importance to evaluate the cause of pancytopenia and planning further investigations. [2] The final interpretation requires the integration of peripheral blood findings, bone marrow aspirate and trephine biopsy evaluation, together with the results of supplementary tests such as immunophenotyping, cytogenetic analysis and molecular genetic studies as appropriate. [3] Bone marrow examination is indicated in all cases of

pancytopenia where the underlying cause is not quite obvious. This is particularly needed in case of hypoplasia/aplasia and to exclude leukaemia or other malignant infiltration. [4]

Varieties of hematological and non-hematological disorders may affect bone marrow either primarily or secondarily, resulting in the manifestation of pancytopenia. [5] The incidence of various haematological disorders causing pancytopenia varies due to geographical distribution and genetic predisposition. [6] New-onset pancytopenia can sometimes result from nutritional deficiency. Copper deficiency, which can occur because of long-term total parenteral nutrition, gastrointestinal surgery, weight reduction surgery, excessive zinc intake, and even renal failure, can lead to hematologic abnormalities, including pancytopenia. [7-9] Pancytopenia occurs late in the course of HIV infection. [10] Chloramphenicol contributed to as many as 20-30% of the cases of drug induced pancytopenias in the recent past. [11,12] Therefore it is required to study peripheral film in cases of pancytopenia. Hence the present study was done to correlate the peripheral blood film and bone marrow findings in cases of pancytopenia.

MATERIAL AND METHOD

The present study was a cross sectional study conducted in the Pathology department of Government Medical College, and Rajindra Hospital, Patiala, Govt. Medical College and Hospital, Amritsar under Professor & Head of Pathology Dr. Arun Puri. Total 60 patients both indoor and outdoor who presented with relevant clinical findings of pancytopenia were taken up for the study. The age and sex of the patients was no criterion for selection of cases. Criteria for diagnosis of pancytopenia considered were Hb less than 10g/dl, TLC less than 4×103/µl and Platelet count less than 1lakh/cumm. The subjects were included and excluded according to the following criteria:

Inclusion Criteria:

1. All patients presenting with symptoms of pancytopenia irrespective of age and gender.

Exclusion Criteria:

1. Patients unfit for Bone Marrow aspiration.
2. Already diagnosed cases of pancytopenia or patients undergoing treatment.
3. Patients who refused to give consent for bone marrow aspiration.

Clinical data with reference to the mode of onset, history of any drug in take or exposure to any toxic chemical agents, bone pains, hepatosplenomegaly and lymphadenopathy was recorded. Investigations performed were analysis of haemoglobin, red cell count, total leucocyte count, differential leucocyte count, coagulation profile, platelet count, erythrocyte sedimentation rate, reticulocyte count and peripheral blood film.

Method: Bone marrow aspiration was done by using Salah's bone marrow puncture needle. The preferred site was posterior superior iliac spine. Leishman's stain, which occupies an intermediate position, is widely used in routine and was used in present study.

Bone marrow aspirate smear: A low power scan was done to see whether the material obtained is satisfactory. High power view and oil immersion lens was used to determine cellular morphology and distribution. The smears were assessed for cellularity, differentiation and maturation of erythroid, myeloid and megakaryocytic lineage, M:E ratio, Plasma cells, Lymphocytes and parasites/ abnormal cells/ granulomas/ storage cells.

Preparation of a peripheral blood smear:

a. **Direct Finger Prick Method:** This method is commonly used in infants and if amount of blood will be less. In adults blood was obtained from distal digit of ring or middle finger. In infants it was collected from great toe or lateral /medial aspect of plantar surface of heel. The puncture site was cleansed with a suitable disinfectant. After drying, a puncture, sufficiently deep to allow free

flow of blood was made with a sterile, dry and disposable lancet. First drop was discarded. Next few drops were collected. After collection, a sterile cotton swab was put and pressed over the puncture site till bedding stopped.

b. From Venous Blood: Blood was collected from the antecubital vein for various hematological and biochemical investigations. A drop of blood was taken from the same syringe and was placed on a clean glass slide 1cm from the end. Another slide with a smooth edge (spreader) was taken and the edge was placed over the drop so that the blood spread along the edge. The spreader was kept at approximately 30 to 40 degree angle and a smear was made with forward movement of spreader. About 2.5 to 3.5 cm length tongue shaped smears were made. At least 2 smears were prepared. The smears were air dried and stained with Leishman's stain.

Statistical analysis: Data so collected was tabulated in an excel sheet, under the guidance of statistician. The means and standard deviations of the measurements per group were used for statistical analysis (SPSS 22.00 for windows; SPSS inc, Chicago, USA).

OBSERVATIONS

In our study there were 36 males (60%) and 24 females (40%) out of total 60 patients showing male predominance. The mean age being 35.62±21.97 years and the total range being 1-90 (table 1).

Table 1: Gender and age distribution among the study subjects

Variables	Value
Male (N, %)	36 (60%)
Female (N, %)	24 (40%)
Age (Mean±SD)	35.62±21.97

Table 2: Mean distribution of haemoglobin,

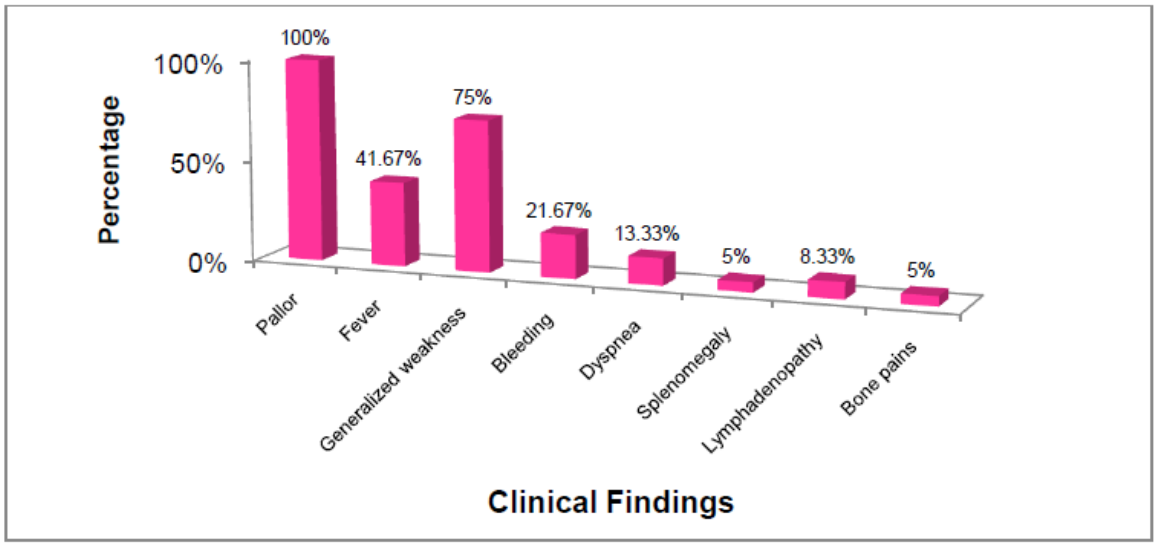
Variables	Value
Haemoglobin (in g/dl)	4.94±1.72
Range of TLC (/cmm)	2827.50±973.26
Range of Platelet Count(/cmm)	50093.33±29205.65
Range of ReticulocyteCount	1.96±1.02

Table 3: Distribution of cellularity on bone marrow and bone marrow hyperplastic lineage

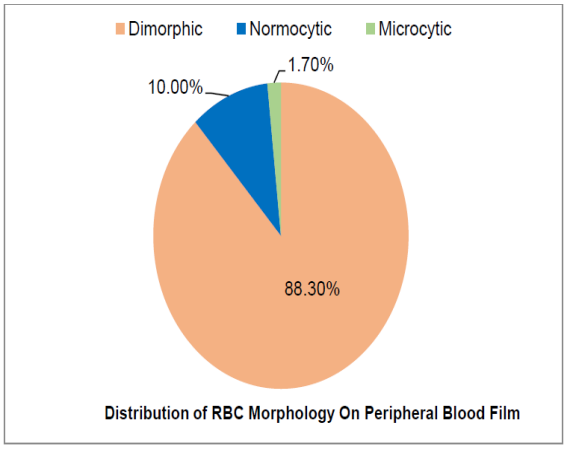
Parameters	N	%
Cellularity		
Normocellular	2	3.38
Hypocellular	9	15.25
Hypercellular	48	81.35
Bone Marrow Hyperplastic Lineage		
Erythroid Hyperplasia (EHP)	46	76.67
Myeloid Hyperplasia (MHP)	2	3.38
Plasmacytosis (PCT)	3	5
Lymphocytic Hyperplasia (LCT)	1	1.67
Blasts (>30%)	1	1.67

Table 4: Correlation of PBF and BMA findings in various causes of Pancytopenia

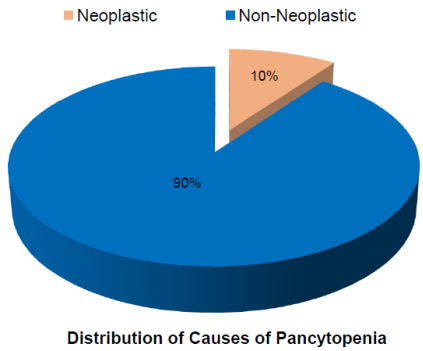
Diagnosis (n=60)	PBF Findings	Bone Marrow aspiration findings	No. of Patients	%age
Megaloblastic anemia (n=46)	Macro+Normo+Ovalo+HSN	Hypercellular with EHP	24	52.17%
	Macro+Normo+Ovalo	Hypercellular with EHP	11	23.91%
	Macro+Normo+HSN	Hypercellular with EHP	11	23.91%
Aplastic anemia (n=6)	Macro+Normo+Ovalo	Hypocellular marrow	5	(83.30%)
	Normocytic Normochromic	Hypocellular marrow	1	(16.70%)
MDS (n=2)	Normo+Macro+HSN	Hypocellular with MHP	1	(50%)
	Normo+Macro+HSN	Hypercellular with MHP	1	(50%)
IMF (n=1)	Normo+ tear drop cells+ nRbc +STL	Dry tap	1	(100%)
Multiple Myeloma (n=1)	Normo+Plasma cells+ Rouleaux +nRBC	Hypercellular with PCT	1	(100%)
Drug induced AA (n=1)	Normocytic Normochromic	Hypocellular	1	(100%)
Acquired AA (HIV) (n=1)	Normo+nRBC	Hypercellular with PCT+LCT	1	(100%)
Acute Leukaemia (n=1)	Micro+Blasts	Hypercellular with Blasts 80%	1	(100%)



Graph 1: Clinical presentation of pancytopenia



Graph 2: Distribution of RBC Morphology on Peripheral Blood Film



Graph 3: Distribution of Causes of Pancytopenia

Pallor was observed in all the patients. Generalized weakness in 45 patients (75%) followed by 25 patients (41.67%) of fever,13 patients (21.67%)of bleeding, 8 patients (13.33%) of dyspnea, 5 patients (8.33%) of lymphadenopathy and 3 patients (5%) of each splenomegaly and bone pains (graph 1).

The mean range of Hb level observed was 4.94±1.72 and the total range observed was 2.0-9g/dl. The mean calculated was 2827.50±973.26 and the total range was 1000-3950/cmm (table 2).

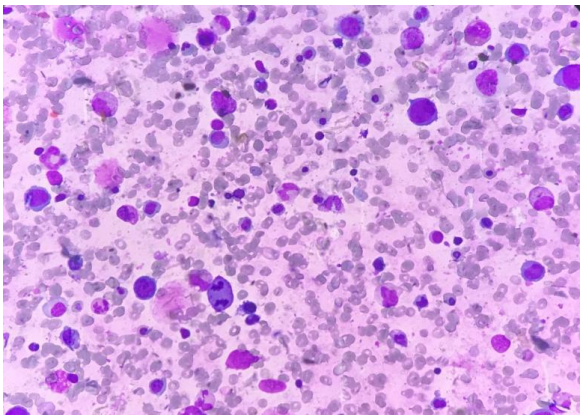


Figure 1: Leishman's Stained Bone Marrow Aspirate Showing Megaloblastic Reaction (x400)

Out of 60 patients, the predominant RBC picture was dimorphic in 53patients (88.30%) followed by normocytic in 6 patients (10%) and microcytic in1 patient (1.70%) as shown in graph 2.

48 patients (81.35%) had hypercellular marrow followed by 8 patients (15.25%) of hypocellular marrow and 2 patients (3.38%) of normocellular marrow. One of the aspirate was dry tap on marrow. In the present study, 46 patients (76.67%) had erythroid hyperplasia, followed by 3 patients (5%) of plasmacytosis, 2 patients (3.33%) of myeloid hyperplasia and 1

patient (1.67%) of each Lymphocytic Hyperplasia and Blasts (>30%) as shown in table 3.

In the present study, the numbers of non- neoplastic causes of pancytopenia were 54 (90%) cases and numbers of neoplastic causes were 6(10%) patients out of total 60 cases (graph 3).

In the present study, total 46 (100%) patients of megaloblastic anemia had macrocytes on peripheral blood film and 35 patients (76%) had hypersegmented neutrophils correlated to bone marrow aspiration which showed hypercellular marrow with erythroid hyperplasia in total 46 patients of megaloblastic anemia (table 4, figure 1).

DISCUSSION

Pancytopenia is commonly encountered in hematology. It occurs in various diseases. The present study analyzed the clinical, hematological and etiological profile of total 60 cases of pancytopenia. Patients were subjected to peripheral blood film examination and bone marrow aspiration.

Present study showed that the range for age of patients was between1 to 90 years which was comparable to the studies conducted by Gayathri et al [13] (2011), Manzoor et al [14] (2014), Vaidya et al [15] (2015) and Biradar et al [16] (2016).Present study showed male preponderance with male to female ratiobeing 1.5:1 which was comparable to the studies conducted by Manzoor et al, [14] Biradar et al [16] and Sahay et al [17] (2018). Out of 60 patients in this study number of males were 36(60%) and number of females were 24(40%) which was comparable to the study conducted Manzoor et al [14] (2014) that showed 31 males and 19 females out of 50 patients. Gayathri et al [13] (2011) also showed 57 males and 47 females out of total 104 patients.

In our study, the most common presentation observed was pallor in all the patients followed by generalized weakness in 45 patients (75%) and fever in 25 patients (41.67%). This was comparable to the study conducted by Manzoor et al [14] which showed that progressive pallor was the most common clinical feature and it was found in almost every case followed by generalized weakness, fever with or without chills, and dyspnea. Biradar et al [16] (2016) concluded that the commonest presenting complaint was fever in 20 (66.6%) cases followed by fatigue in 12(40%) patients while pallor was present in all the patients.

The mean Hb level calculated was 4.9g/dl and the peak range calculated was 4 to 5.9g/dl in the present study. It was in concordance with the study by Biradar et al [16] (2016) which showed peak range of Hb as 5-8g/dl and 5.1-7g/dl (41.0%) respectively. In our study the peak range of TLC observed was 2501-4000/cumm and the mean calculated was 2827.50/cumm. Lowest TLC observed in AA as1100/cumm. This was comparable to the study conducted by Kulkarni et al [18] (2017) which revealed maximum number of patients with TLC in the range2001-3999/cumm. Similar results were obtained from the study conducted by Gupta et al [19] (2016) which revealed that mean TLC 2.14 ×109/L. Lowest observed in MDS 1090/cumm.

In the present study out of 60 patients, the predominant RBC picture was dimorphic in 53 patients (88.30%) followed by normocytic in 6 patients (10%) and microcytic in 1 patient (1.70%).The study by Patel et al [20] (2018) was in contrast to our study as the observed predominant blood picture was normocytic normochromic (38.5%) followed by dimorphic (32.7%), macrocytic (17.3%) and microcytichypochromic (5.8%). In a study by Agarwal et al [21] (2018) it was concluded that macrocyticblood picture was seen in 45% of cases. Microcytosis was noticed in 10%cases while 28% cases showed normocytic picture. 17% cases had dimorphic picture.

In this study, the maximum number of patients i.e. 48 (81.35%) showed hypercellularity on Bone Marrow. Hypocellular Marrow was observed in 9

patients (15.25%). Normo cellular marrow was observed in 2 patients (3.38%). Erythroid Hyperplasia being observed in majority of cases 46(76.67%). In the study by Javalgi et al [22] (2013), 73 cases (68.8%) had hypercellular marrow, followed by normocellular (15%) and hypocellular marrow (16.2%). Normocellular marrow in pancytopenic patients can be the result of sequestration or destruction of cells by the action of antibodies and trapping of normal cells in a hypertrophied and over-reactive reticuloendothelial system.

In the present study, the numbers of non- neoplastic causes of pancytopenia were 54 (90%) cases and number of neoplastic causes were 6(10%) patients out of total 60 cases. This was comparable to the study by Sharif et al [23] (2014) which showed 92/105 (88%) cases as non neoplastic causes of pancytopenia and13/105 (12.38%) cases as neoplastic causes.

In our study, out of 46 patients, 35 patients (76%) of megaloblastic anemia showed hypersegmented neutrophils on peripheral blood. Macrocytes were seen in all the cases of megaloblastic anemia on peripheral blood. Microcytic picture along with blasts was observed on PBF which correlated with bone marrow aspiration which showed hyper cellular marrow with 80% blast in case of acute leukaemia. Normocytic picture was observed on PBF correlated with bone marrow aspirate which showed hypocellular marrow in patients of aplastic anemia and drug induced aplastic anemia. Agarwal et al [21] (2018) concluded that the most common finding on peripheral blood smear was anisocytosis seen in 94% cases followed by macrocytic blood picture (45%). Hypersegmented neutrophils were seen in80% cases of megaloblastic anemia. Circulating blasts were seen in 18.75%cases, which included 9 cases of acute leukemias. Various factors such as differences in methodology geographic distribution, genetic disturbances and period of observation may cause variation in the incidence of disorders causing pancytopenia. [24-25]

CONCLUSION

Pancytopenia is very commonly encountered in clinical practice. Peripheral blood film findings along with clinical examination help to plan investigations and give important diagnostic clues. In cases where clinical findings are masked and peripheral blood film does not provide clue to diagnosis bone marrow aspiration becomes necessary. There is a significant correlation between bone marrow aspiration findings and peripheral blood film findings. Both the investigations are complementary to each other in evaluating the patient. The causes of pancytopenia show a wide variation due to differences in period of observation, geographic area, exposure to cytotoxic agents and differences in genetics. A comprehensive approach including clinical and hematological study of patients with pancytopenia help in diagnosing the cause.

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