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Original Research Article

A Cross Sectional Study of Bone Marrow Examination in Patients with Pancytopenia and with Respect to Iron Stores in the Bone Marrow

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Abstract

Background: Pancytopenia refers to decrease in all three hematologic cell lines in the peripheral blood below the normal reference range. Haematopoietic and non-hematopoietic conditions manifest with features of pancytopenia and hence marrow composition will differ. The severity of pancytopenia and underlying pathology determines the management and prognosis of these patients [1].

Objective: The current study was undertaken to evaluate the common causes of pancytopenia by correlating haematological and bone marrow findings and also studying the grading of iron store, with an intent for early diagnosis and appropriate treatment to patients with pancytopenia.

Methodology: A prospective study was conducted at a tertiary hospital in Maharashtra in 50 patients detected as Pancytopenia, based on peripheral smear examination with haemoglobin <10 mg/dL, total leukocyte count <4000 cells/mm^3 and platelet count <150,000 cells/mm^3. Bone marrow aspiration was done and Prussian blue stain was used to assess the iron stores in bone marrow aspirates.

Results: 48% of patients had haemoglobin<5gm/dl, 78% had leucocyte count of1-3x10^3/mm^3 and 50% had Platelet count of 50-100 x10^3/mm^3. Megaloblastic anaemia was the commonest cause of Pancytopenia (66%) followed by Subleukemic leukemia, dry tap, Dimorphic Anaemia, Iron deficiency anaemia and Multiple myeloma. Out of 33 cases of Megaloblastic anaemia,18 showed normal iron stores, while 15 showed increased iron stores. Decreased iron stores were seen in dimorphic anaemia and iron deficiency anaemia.

Conclusion: Megaloblastic anaemia is a common cause of Pancytopenia and bone marrow study is a useful tool in diagnosis of pancytopenia. Prussian blue stain helps to diagnose co-existence of iron deficiency in other anaemias.

Keywords: Pancytopenia, Megaloblastic anaemia, Peripheral Blood Examination, Bone Marrow study, Perls' stain.

Introduction

Pancytopenia is the simultaneous presence of anaemia, leukopenia and thrombocytopenia [2]. Therefore, it exists in adults, when Haemoglobin [Hb] is less than 13.5 g/dl (males) and 11.5g/dl (females), the leucocyte count is less then $4x10^3$/dl and the platelet count is less than$150x10^3$/dl [3]. The presenting symptoms can be due to anaemia, leukopenia or thrombocytopenia. Common clinical manifestations are pallor, fatigue, splenomegaly, lymphadenopathy, fever, bleeding, weight loss, hepatomegaly and jaundice. Thrombocytopenia can lead to bruising and mucosal bleeding. Leukopenic features, though uncommon as the presenting symptom, may sometimes become a life-threatening condition. [3]

Pancytopenia may be a manifestation of a wide variety of disorders, which primarily or secondarily affect the bone marrow. The study of bone marrow failure was first done by Paul Ehrlich in 1888, in a young woman who died from severe anaemia, bleeding and fever. As a pathologist, Ehrlich was struck by the absence of nucleated RBCs and the fatty quality of the femoral marrow. [4]. Variety of haematopoietic and non-hematopoietic conditions manifest with features of pancytopenia. The cause of pancytopenia may thus lie in the bone marrow, periphery or both. Causes of pancytopenia include Hypocellular bone marrow-associated causes e.g. Aplastic anaemia, Cellular bone marrow disease such as Leukaemias, Lymphomas, or Myelodysplastic syndromes, Multiple myeloma, Metastases, and Cellular marrow with systemic disorders due to Vit B12 or folate deficiency, infections, autoimmune disorders, etc.[3].

It may be due to ineffective erythropoiesis, decreased cell production, increased peripheral utilization and increased destruction without an adequately compensatory increase in the cell production. The cause of pancytopenia may thus lie in the bone marrow, periphery or both. Marrow cellularity and composition differ in relationship to the cause. The marrow is generally hypocellular in pancytopenia caused by a primary production defect. Pancytopenia resulting from ineffective haematopoiesis, increased peripheral utilization or destruction of cells and infiltration in bone marrow usually have a normocellular or hypercellular marrow.

Cause of pancytopenia may be from a simple treatable disease to a serious life-threatening condition. The severity of pancytopenia and underlying pathology determines the management and prognosis. Hence, it is important to evaluate these patients to provide early diagnosis and timely correct treatment.[1]

Objective

To evaluate the common causes of pancytopenia by correlation of haematological and bone marrow findings and also study the grading of iron stores in such conditions.

Materials and Methods

A cross-sectional study of 50 patients with pancytopenia detected on peripheral blood examination was undertaken from January 2021 to November 2022 at our Tertiary Health care hospital after obtaining due approval from the Ethical committee of the institution. It was a prospective study in patients between 14 to 79 years of age. The study population was directed to include cases of Pancytopenia and also detection of iron stores in the bone marrow aspirates. Sample size was calculated by using data from a study conducted by Dr. Deepa Tekwani et al [5] Using statistical formula, Sample size = Z2xpxq/n2, where z=1.96, p is

prevalence, q is (100-p), n is absolute precision value (0.2), the minimum required sample size was calculated as 45, and was rounded off to 50.

Case selection was based on clinical features, physical findings and supported by laboratory tests for Complete blood count, using automated 5-part haematology analyser HORIBA (YUMIZEN H550) and peripheral blood smear examination with Leishman stained smears.

Inclusion Criteria: All patients with reduced three cell lines, Haemoglobin less than 10g/dl (keeping in mind the average level in our community), leucocyte count less than $4x10^3/mm^3$and platelet count less than $150x10^3/dl$,

Exclusion Criteria: All diagnosed cases of cancer on radiotherapy and or chemotherapy, patients with reduction in only one/two blood cell lines, patients suffering from bleeding disorders and patients suffering from bone disorders. Bone marrow aspiration and/or biopsy was subsequently carried out after obtaining the written consent. Perls' Prussian blue stain was done to assess the iron stores in bone marrow aspirates. The collected data was tabulated and analysed in Microsoft excel.

Results

Most common aetiology detected was Megaloblastic anaemia (33 cases/ 66%), followed by Subleukemic Leukemia (7 cases/ 14%). Other causes of pancytopenia were Dimorphic anaemia (3 cases/6%), Dry tap (3 cases/6%), Iron deficiency anaemia (2 cases/ 4%) and Multiple Myeloma (2 cases/ 4%) **[Table1].**

Table 1: Aetiological distribution of cases of pancytopenia

Cause of Pancytopenia	Number of cases	Percentage (%)
Megaloblastic Anaemia	33	66
Subleukemic Leukemia	7	14
Dimorphic Anaemia	3	6
Dry tap	3	6
Iron Deficiency Anaemia	2	4
Multiple Myeloma	2	4
Total	50	100

The study population consisted of 50 patients with age range of 14 to 79 years. Most of the patients were in the age group of 16-30 years (40%) and least number of cases (3) were seen beyond 60 years, almost similar to results of the study by Akshatha Savith *et al* [6], with age from 15 to 75 years **[Table-2].**

Table 2: Age wise distribution in various causes of Pancytopenia

Causes of pancytopenia	Age in years					Total
	<15	16-30	31-45	46-60	>60	
Megaloblastic Anaemia	2	17	9	5	-	33
Subleukemic Leukemia	1	-	3	2	1	7
Dimorphic Anaemia	1	1	-	1	-	3
Dry tap	-	1	2	-	-	3
Iron deficiency anaemia	-	1	-	1	-	2
Multiple myeloma	-	-	-	-	2	2
Total	4	20	14	9	3	50

Both males and females were commonly affected by Megaloblastic anaemia with 23 and 10 cases respectively. Iron deficiency anaemia and Dimorphic anaemia was seen in females, with only 1 case of the latter in males. **Subleukemic** leukemia and dry tap was commoner in the males than females (2:1). Out of 50 patients, 31 (62%) were males and 19 (38%) females in the study group with male: female ratio of 1.6:1. Males were most commonly affected in all age groups. Gayathri and Rao *et al* [7], also found higher incidence in males (54.81%) compared to females (45.19%), with male-to female (M: F) ratio of 1.2: 1**[Table3].**

Table 3: Gender distribution for various causes of Pancytopenia

Cause of Pancytopenia	Males	Females	Total
Megaloblastic Anaemia	23	10	33
Subleukemic Leukemia	5	2	7
Dimorphic Anaemia	1	2	3
Dry tap	2	1	3
Iron Deficiency Anaemia	-	2	2
Multiple Myeloma	-	2	2
Total	31	19	50

Peripheral blood examination revealed severe reduction in haemoglobin concentration <5 g/dl in 24 cases (48%), moderate fall between 5.1-8g/dl in 23 cases (46%) and mild reduction between 8.1-10g/dl in 4 cases (8%).

Severe decrease in haemoglobin was seen in Megaloblastic anaemia (16 cases), Subleukemic leukemia (3 cases), Dimorphic anaemia (3 cases) and Multiple Myeloma (2 cases). Moderate decrease was observed in Megaloblastic anaemia (13 cases), Subleukemic leukemia (4 cases) and Dimorphic anaemia (2 cases), dry tap (2 cases) and Iron deficiency anaemia (1 case). Mild decrease in haemoglobin concentration was observed in 1 case each of Megaloblastic anaemia, Dimorphic anaemia, dry tap and Iron deficiency anaemia. Thus, severe to moderate decrease in haemoglobin concentration was mainly seen in Megaloblastic anaemia (29), Subleukemic leukemia (7) and Dimorphic anaemia (5).

The total leukocyte count was severely decreased (<1000 cells/mm^3) in 5 cases (10%), in 2 cases each of Megaloblastic anaemia and Subleukemic leukemia and 1 case of Iron deficiency anaemia. Moderately decreased WBC count (1000-3000cells/mm^3) was observed in total 39 cases (78%) comprising of Megaloblastic anaemia (29), Subleukemic leukemia (3), Dimorphic anaemia (3), Dry tap (3) and Multiple Myeloma (1). Mildly decreased WBC count (3001- 4000cells/mm^3) was observed in 6 cases (12%), comprising of Megaloblastic anaemia (2), Subleukemic leukemia (2), Iron deficiency anaemia (1)and Multiple Myeloma (1).

Platelet count showed severe reduction (<20000/mm^3) in 7 cases (14%) of Megaloblastic anaemia (5) and Subleukemic leukemia (2) while it was moderately reduced (20,001-50,000/mm^3) in 18 cases (36%) with Megaloblastic anaemia (12), Subleukemic leukemia (2), Dimorphic anaemia (2), Dry tap (1) and Multiple Myeloma(1). Mild decrease in Platelet count (50,001-1,00,000/mm^3) was seen in 25 cases (50%) with Megaloblastic anaemia (16), Subleukemic leukemia (3), Dimorphic anaemia (1), Dry tap (2), Iron deficiency (2) and Multiple Myeloma (1).

The peripheral smear finding most commonly observed was Macrocytic hypochromic blood picture in 26 cases of

Megaloblastic anaemia, while macrocytic normochromic blood picture was seen in 8 cases with Megaloblastic anaemia (7) and Subleukemic leukemia (1). Dimorphic hypochromic appearance was seen in 8 cases, Subleukemic leukemia (3), Dimorphic anaemia (3) and Dry tap (2). Microcytic hypochromic blood picture was seen in 4 cases, Subleukemic leukemia (2) and Iron deficiency anaemia (2). Normocytic normochromic blood picture was seen in 4 cases of Multiple myeloma (2), Dry tap (1) and Subleukemic leukemia (1).

Bone Marrow aspirates were evaluated for cellularity- **Table 4**

Observations were a hypercellular marrow (43 cases, 86%)- **Fig 1**. Hypocellular marrow was seen in 3 cases (6%) and normocellular marrow in 1 (2%). 3 cases (6%) could not be commented upon due to diluted marrow/ dry tap. Hence, bone marrow biopsy was advised for further evaluation.

Table 4: Bone marrow cellularity

Cellularity	Frequency	Percentage (%)
Hypercellular	43	86
Normocellular	1	2
Hypocellular	3	6
Diluted marrow/dry tap	3	6
Total	50	100

Erythroid hyperplasia was dominant in 36 cases (72%). M:E ratio could not be commented upon in 3 cases (6%) due to diluted marrow/dry tap on aspiration. Erythroid hyperplasia with megaloblastic maturation was the commonest finding in 33 cases (66%) -**Fig 2.** Mixed erythroid hyperplasia was seen in 3 cases (6%) with both micronormoblasts and megaloblasts. Normal erythroid maturation with normal cellularity was seen in 1 case (2%) and normoblastic hyperplasia seen in Iron deficiency anaemia (1 case). Hypercellular marrow with myeloma cells was seen in 2 cases of Multiple Myeloma- **Fig 3** and increased myelopoiesis noted in 7 cases of Subleukemic leukemia with Myeloblasts in 2 and lymphoblasts in other 5. Normal megakaryocytic maturation was observed in 33 cases. Increased numbers of megakaryocytes were noted in 7 cases of Megaloblastic anaemia while hypoplasia was seen in 7 cases, in Subleukemic leukemia (4) and Dimorphic anaemia (3).

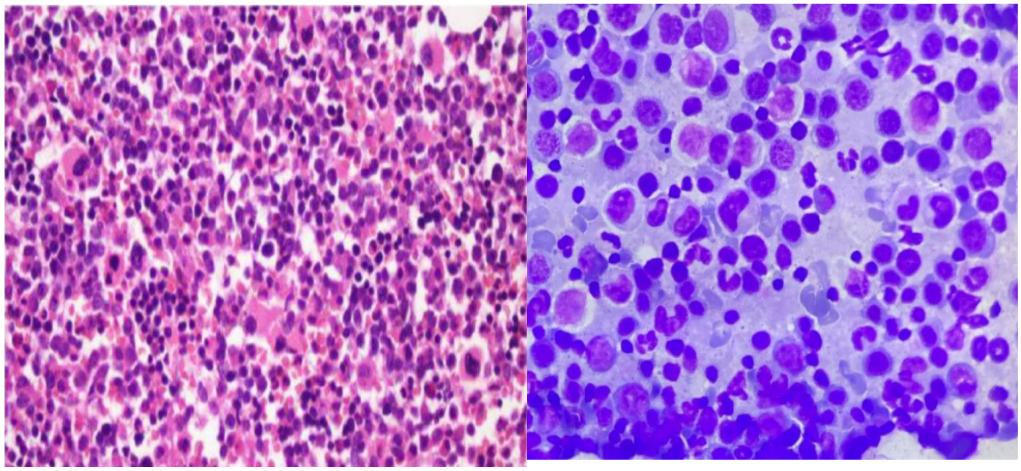


Figure 1: Hypercellular Marrow (Leishman stain 40x)
Figure 2: Bone Marrow in Megaloblastic Anaemia (Leishman stain 40x)

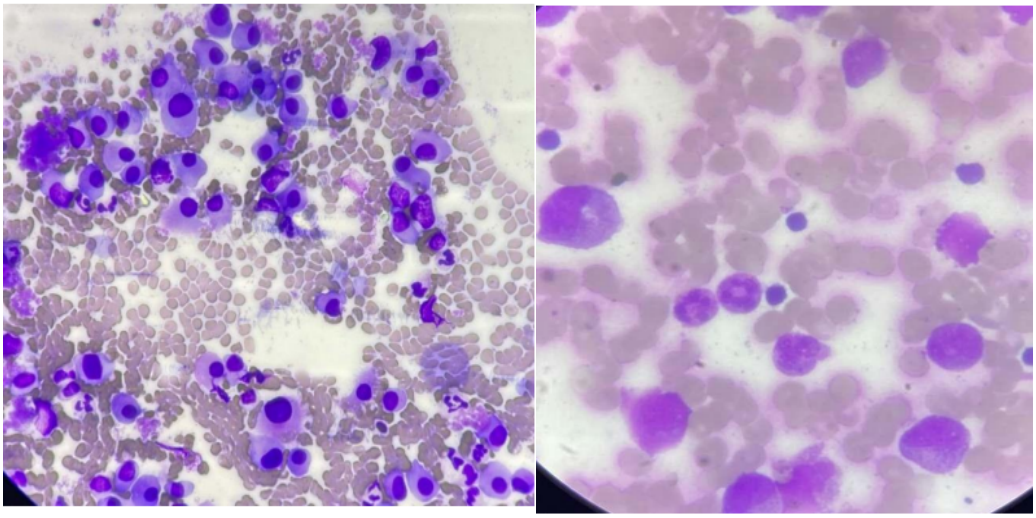
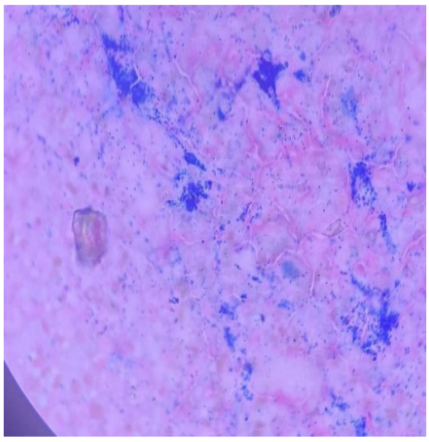


Figure 3: Bone Marrow in Multiple Myeloma (Leishman stain 40x)

Figure 4: Bone Marrow in Subleukemic Leukemia (Leishman stain 40x)



Perls' Prussian blue stain was done to assess the iron stores in bone marrow aspirates-**Table 6**. Iron stores were normal in most cases (33 cases, 66%), low in 3 cases (6%), high in 11 cases (22%) and could not be commented in 3 cases (6%) due to diluted marrow/dry tap. Most cases of Megaloblastic anaemia (18 cases) showed normal iron stores and 15 cases showed increased iron stores in the present study- **Fig 4** Decreased iron stores were seen in dimorphic anaemia and iron deficiency anaemia.

Figure 4: Bone Marrow with Iron Grade 6+ (Prussian Blue)

Table 5: Grading of bone marrow iron stores in various causes of pancytopenia.

	Megaloblastic anaemia	**Subleukemic Leukemia**	**Dimorphic anaemic**	**Dry tap**	**Iron Deficiency anaemia**	**Multiple myeloma**
1+	-	-	2	-	1	-
2+	7	1	1	-	1	-
3+	11	3	-	-	-	-
4+	7	1	-	-	-	-
5+	5	2	-	-	-	2
6+	3	-	-	-	-	-
Dry tap	-	-	-	3	-	-
Total	33	7	3	3	2	2

Discussion

A total of 50 cases of pancytopenia were studied. Age, gender, peripheral blood picture and bone marrow aspiration smears were analysed for various causes of pancytopenia and observations were compared with other studies published in the literature.

The age of the patients in the present study ranged from 14 to 79 years, with a male preponderance of 31 males (62%) and 19 females (38%), thus the male-to-female (M: F) ratio was 1.6: 1. which is comparable to a study by Kumar et al [8], with an age range from 12 to 73 years and M: F ratio of 2.1:1. Similar observations were noted by Santra G et al [9], with age between 13-65 years and M: F ratio of 1.5:1.

Majority of the patients had haemoglobin <5gm % (48%), 78% of the patients had leucocyte values in the range of 1001-3000 cells/$mm^3$and platelet count in the range of 50-100x10^3/mm^3 was seen in the majority (50%) which compares with the study by Akshatha Savith et al [6] who found haemoglobin to be 5-8 gm/dl in 52% of patients, total leukocyte count between 2-4x10^3/mm^3, 82% of patients and Platelet count <50x10^3/mm^3in 50% patients. Pereira and Dias found Haemoglobin in majority between 5.1-7 g/dl (41.0%), WBC count of 3.1-4x10^3/mm^3 (37.5%) and platelet count of 76-100x10^3/mm^3 (78%) [10].

Megaloblastic anaemia was the commonest cause (66%), followed by Subleukemic leukemia (14%), Dimorphic anaemia (6%), Dry tap (6%), Iron deficiency anaemia (4%) and Multiple Myeloma (4%). Rajesh and Shailaja Para in a study of 58 cases reported Megaloblastic anaemia (46.6%), Subleukemic leukemia (3.4%) and 5.2% of Iron deficiency anaemia[11].

Bone marrow aspiration study showed predominantly megaloblastic erythroid hyperplasia (66%) followed by Subleukemic leukemia (14%), Dimorphic Anaemia (6%), Iron deficiency anaemia (4%), Multiple myeloma (4%), dry tap (6%). Biradar et al in a study of bone marrow aspirates in 30 cases of pancytopenia, found megaloblastic anaemia as the commonest cause (46.6%), the uncommon causes being Acute leukemia, Myelodysplastic Syndrome, Myelofibrosis and Multiple Myeloma[12].

In the present study, there were 2 cases of multiple myeloma and Jha et al in their study of 148 cases reported one case of plasma cell myeloma [13]. Kishore Khodke et al [14] reported 2 cases of multiple myeloma.

Perls' stain for Iron study revealed 33 (66%) cases with macrocytic anaemia having iron grade ranging from 2-6 and similar findings were observed in Pujara et al [15]. Chanarin et al in a study of 127 cases of megaloblastic anaemia, found 37 cases (29.13%) with absent iron stores and ascribed this to iron deficiency with nutritional cobalamine deficiency [16].

Our study had 3 cases of dimorphic anaemia (6%), with iron grade ranging from 1-2. Rajeev Saxena et al found iron grade of 1-4 in their study with 15 cases (13.63%) of dimorphic anaemia [17].

Our study had 1 case of microcytic anaemia with iron grade of 1 which was similarly observed by Pujara et al [15], Deka et al [18]and Alexander et al [19]. In present study, the other case of microcytic anaemia had iron grade of 2, having received parenteral iron therapy before the bone marrow study, comparable to study of Jameson et al [20], with 4 cases of iron deficiency anaemia receiving parenteral iron before bone marrow study with iron storage grade of 2 and 3.

Conclusion

Pancytopenia is not an uncommon haematological problem and should be suspected when a patient presents with unexplained anaemia, prolonged fever and tendency to bleed. The clinical findings and peripheral blood film reveal the most probable cause of anaemia. Presence of nucleated RBCs and/or immature myeloid cells may suggest marrow infiltration or primary hematologic disorder. Megaloblastic anaemia was the commonest cause of pancytopenia in the present study and was similar to many other Indian studies, such as by Kumar et al [21], but most studies done outside India have reported aplastic anaemia as the commonest cause. Metikurke S, et al [22], in their study of 58 cases of pancytopenia with Bone Marrow aspirates found 25% patients between 21-30 years, male preponderance (60%) and Megaloblastic anaemia as the commonest cause [39.6%], similar to our study, followed by nutritional anaemia [24.1%] and aplastic anaemia [12.06%]. The study had a limitation in the 3 cases of Dry tap where a trephine biopsy would have been diagnostic, but could not be performed due to patient non-compliance. We conclude that bone marrow examination is a single useful investigation which reveals the underlying cause in patients with pancytopenia and is sufficient in making diagnosis of Megaloblastic anaemia and initial diagnosis of leukaemia, myeloma, metastatic deposits in the bone marrow, etc. Prussian blue stain is a simple and helpful technique to measure body iron semi-quantitatively and to diagnose coexistence of iron deficiency with anaemia due to other causes. Fortunately, Megaloblastic anaemia, as a cause of Pancytopenia reflects the higher prevalence of nutritional anaemia in the Indian subjects which responds very well to treatment and hence the importance of early diagnosis with appropriate hematological techniques for timely treatment and improving patient well-being.

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