Original Article

PATHOPHYSIOLOGY OF PANCYTOPENIA: UNDERSTANDING BLOOD CELL PRODUCTION AND REGULATION IN THE BONE MARROW

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ABSTRACT

Objective: The bone marrow is the primary site for blood cell formation, responsible for the production and export of red cells, platelets, and granulocytes. Pancytopenia is a condition characterized by a reduction in all three types of cellular components in peripheral blood, leading to symptoms of marrow failure. The management and prognosis of pancytopenia depend on identifying the underlying pathology.

Methods: The pathophysiology of pancytopenia involves various factors such as infections, toxins, malignant cell infiltration, suppression, or ineffective hematopoiesis. Bone marrow examination, including aspiration and trephine biopsy, is crucial for diagnosing pancytopenia, especially when the cause is unclear or when malignancy is suspected. Geographic distribution and genetic disturbances contribute to variations in the incidence of disorders causing pancytopenia.

Results: The study aims to evaluate the various causes of pancytopenia and correlate peripheral blood findings with bone marrow aspirate and trephine biopsy. The collected data will assist in planning the diagnostic and therapeutic approach for patients with pancytopenia.

Conclusion: Pancytopenia involves disruptions in blood cell production and regulation. Bone marrow aspiration and trephine biopsy are crucial for diagnosis. Understanding blood cell regulation provides insights. This study aims to enhance diagnostic and therapeutic approaches for pancytopenia.

Keywords: Pancytopenia, Bone marrow, Peripheral blood

INTRODUCTION

The bone marrow, the largest and widely distributed organ in the body serves as the primary site for blood cell formation [1]. It plays a critical role in the daily production and export of billions of red cells, platelets, and granulocytes per kilogram of body weight [2]. However, various disorders affecting the bone marrow can lead to a condition known as pancytopenia, characterized by a reduction in all three types of cellular components in peripheral blood, namely anemia (reduced red blood cells), neutropenia (reduced white blood cells), and thrombocytopenia (reduced platelets) [3]. Individuals with pancytopenia may experience symptoms of marrow failure, such as pallor, dyspnea, bleeding, bruising, and increased susceptibility to infections [4]. The management and prognosis of pancytopenia depend on identifying and addressing the underlying pathology [5].

The pathophysiology of pancytopenia is multifactorial. It can occur due to decreased production of hematopoietic cells in the bone marrow caused by factors like infections, toxins, malignant cell infiltration, suppression, or ineffective hematopoiesis [6]. Additionally, pancytopenia can result from dysplasia, maturation arrest of all cell lines, or peripheral sequestration of blood cells [7]. When the cause of pancytopenia is not apparent from the clinical history, a thorough examination of a blood film is crucial [8]. If the cause remains unclear, bone marrow examination becomes essential for accurate diagnosis [9].

Bone marrow aspiration and trephine biopsy are significant procedures in the laboratory workup and diagnosis of pancytopenia. They are commonly performed when pancytopenia is unexplained or when there is suspicion of malignant conditions like leukemia. These procedures also aid in the diagnosis and staging of neoplasms and storage disorders [10]. Trephine biopsy is typically conducted when hypoplasia or aplasia is observed during aspiration. Several factors contribute to variations in the incidence of disorders causing pancytopenia, including geographic distribution and genetic disturbances [11]. While some studies exist in the literature, there is

a lack of extensive discussion about this abnormality in major textbooks of internal medicine and hematology.

The objective of the present study is to evaluate the various causes of pancytopenia and correlate peripheral blood findings with bone marrow aspirate and trephine biopsy, if necessary. The data collected will assist in planning the diagnostic and therapeutic approach for patients with pancytopenia. Blood cell production involves a complex process of hierarchical developmental progression of hematopoietic stem cells. These stem cells gradually lose their developmental potentials and commit to a single cell lineage, which eventually matures into specific blood cell types [12]. Hematopoietic stem cells predominantly reside within the bone marrow, in close association with non-hematopoietic cells that make up the bone marrow microenvironment. The progression of hematopoietic stem cells through different stages of stem and progenitor compartments relies on the presence of soluble factors called cytokines [1].

The regulation of erythropoiesis (red blood cell production), granulopoiesis and monopoiesis (granulocyte and monocyte production), megakaryopoiesis (platelet production), and lymphopoiesis (T and B lymphocyte production) involves intricate interactions between various factors, including cytokines and growth factors. Each lineage has its own specific regulation mechanism [2, 9, 10]. For instance, erythropoiesis primarily occurs within the bone marrow, where local cytok release from the bone marrow stroma and binding of cytokines to the stromal matrix play a crucial role in controlling the rate of proliferation and differentiation of erythroid cells [2]. Erythropoietin, primarily produced by the kidneys, stimulates the conversion of erythropoietin-responsive cells to pronormoblasts, facilitating the maturation of red blood cells [2]. Granulopoiesis and monopoiesis, which involve the production of granulocytes and monocytes, originate from a common bipotential stem cell. Macrophages play a vital role in regulating the production of their own precursors and granulocytes by producing Colony Stimulating Factor (CSF) and Prostaglandin E (PGE) [2, 9]. Granulocytes also contain inhibitory substances that contribute to the negative feedback

between the mass of mature granulocytes and the rate of production of new cells [2].

The process of megakaryopoiesis, responsible for platelet production, involves the differentiation of pluripotent stem cells into megakaryocytes under the influence of thrombopoietin (TPO) and cytokines such as IL-3 and IL-11 [2]. Thrombopoietin acts as the master growth factor for thrombopoiesis, facilitating the complete sequence of maturation without the need for additional factors [2]. Lymphopoiesis, which gives rise to T and B lymphocytes, occurs in both primary and secondary lymphoid tissues. Antigen-independent lymphopoiesis takes place in primary lymphoid tissues, including the bone marrow and thymus, where committed lymphoid stem cells differentiate into immunocompetent T and B lymphocytes [2, 11]. Antigen-dependent lymphopoiesis occurs in secondary lymphoid organs, such as the adult bone marrow, spleen, lymph nodes, and gut-associated lymphoid tissue, where immunocompetent lymphocytes respond to antigenic stimulation, leading to the formation of effector T and B lymphocytes [2, 11].

Understanding the intricate regulation of blood cell production in the bone marrow provides insights into the potential disruptions that can lead to pancytopenia. By investigating the causes of pancytopenia and correlating peripheral blood findings with bone marrow examination, this study aims to contribute to the diagnostic and therapeutic approach for patients with this condition. The data collected from this research will assist in the development of more targeted and effective treatments for individuals with pancytopenia, ultimately enhancing their quality of life.

MATERIALS AND METHODS

The present study entitled was carried out over a period of two years from January 2011 to December-2012 on patients attending the outdoor and from indoor department of J. L. N. Medical College and associated group of hospitals. A total of 28 cases of pancytopenia were diagnosed in pathology laboratory of J. L. N. Medical College and associated groups of hospitals, Ajmer were included for the purpose of the study.

A brief clinical history, general physical examination, systemic examination, relevant investigation (if necessary followed by special investigation) were performed. PBS, bone marrow aspiration were performed and whenever available bone marrow trephine biopsy were processed.

Criteria for diagnosis of pancytopenia

• Haemoglobin level less than 13.5 g/dl in males or 11.5 g/dl in females,

• Total leukocyte count less than 4×10^9/l and,

• Platelet count less than 150×10^9/l

Indication of bone marrow aspiration (Singh T, 2011)

• Red cell disorder-Megaloblasticanemia, pure red cell aplasia, pancytopenia

• Leukocyte disorder-acute leukemias, subleukamic and aleukamicleukamias

• Megakaryocytic and platelet disorder-thrombocytopenia and thrmobocytosis

• Myeloproliferative disorder

• Myelodysplastic disorder

• Paraprotienemias-MGUS, multiple myeloma, waldenstrom macro globe mia

• Infections

• Lysosomal or other storage diseases

• Iron store assessment

• Metastasis

• Unexplained hepatomegaly and/or splenomegaly

• Marrow harvesting for transplantation

In all patients, a detailed relevant history, including the treatment history, history of drug intake, radiation exposure carried out. Meticulous clinical examination of every patient was done for pallor, jaundice, hepatomegaly, splenomegaly and lymphadenopathy. After history and examination basic investigations performed for each patient including Haemoglobin, hematocrit value, Total leukocyte count, Platelet count, Reticulocyte count. Absolute values including packed cell volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) calculated for every patient. Blood film examination after staining with Leishman's and Giemsa's stains for red cell morphology carried out. Chest radiograph and abdominal ultrasonography also be done in selected patients. Bone marrow aspiration and trephine biopsy executed in patients wherever required. Immunohistochemical stain and cyto-chemical stain done whenever needed.

Table 1: Following investigations were done

Investigation	Method
Bleeding time	Ivy's method
Clotting time	Tube method
Hemoglobin percentage	Sysmexkx 21
Total leucocyte count	Sysmexkx 21
RBC Count	Sysmexkx 21
Platelet count	Sysmexkx 21
Packed cell volume	Sysmexkx 21
Red cell indices	Sysmexkx 21
Reticulocyte count	New methylene blue

The peripheral smear was studied after staining with Leishman's stain.

Staining technique (Lewis *et al.,* 2006)

Leishman's stain

• Smears were air-dried.

• Slides placed on a slide stand and covered with Leishman's stain for 5 min.

• Double the amount of distilled water added over the Leishman's stain and left for 10 min.

• Smears washed with distilled water and air-dried.

Result: Cytoplasm-Pink; Nucleus–Blue

Giemsa stain

• Smears are thoroughly dried.

• Smears were placed in methanol for 15-20 min.

• Transferred to May-Grunwald staining jar for 15 min.

• Then transferred without washing to Giemsa's stain for 10-15 min.

• Washed in buffered water for 2-5 min.

• Slides are dried and examined.

Cytochemical stain

Myeloperoxidase stain

(Stain composition: Ethanol, Benzidinedihydrochloride, Zinc sulphate, Sodium acetate, Hydrogen peroxide, Sodium hydroxide and Safranin)

• Smears placed in formalin-alcohol fixative for 60 seconds at room temperature.

• Washed in running tap water for 15-30 seconds.

• Excess water removed and stain put and left for 30 seconds.

• Washed in running tap water for 30 to 60 seconds.

- Slides dried and examined.

Results: Nuclei-Red; Peroxidase activity-Blue granules

Periodic acid schiff stain

- Fixed air dried smears for 10-15 min informal in ethanol at room temperature (10 ml of 40 % formalin and 90 ml absolute ethanol).

- Washed in running tap water for 5-10 min.

- Incubated in 1 % periodic acid for 20 min at room temperature.

- Washed in running tap water for 5-10 min.

- Incubated in Schiff reagent at room temperature.

- Washed in running tap water for 10 min.

- Counter-stained with Harris Hematoxylin for 5-10 min.

- Washed in running tap water for 5 min and air dried.

Results: Nuclei-Blue. Substances which show positive reaction: Magenta Pink.

Special stains-Periodic acid Schiff reagent stain, Myeloperoxidase, Sudan black and Perls' stains were used wherever indicated.

RESULTS

Table 2: Clinical features

Symptoms	No. of cases	Percentage
Generalized weakness	13	46.4
Fever	13	46.4
Abdomen distension	1	3.6
Pain abdomen	1	3.6

Generalized weakness and Fever (46.4 %) were the commonest symptom in pancytopenic patients, followed by Abdomen distension and pain abdomen (3.6 %).

Table 3: Range of hemoglobin in patients with pancytopenia

Hemoglobin percentage (g %)	No. of cases	Percentage
3.1-5	2	7.1
5.1-7	13	46.4
7.1-10	13	46.4
Total	28	100

The Hemoglobin percentage varied from 3.8 g % to 9.5 g %. Majority (92.8%) of the patients had values ranging from 5.1 to 10 g %, while only 7.1 % of the patients had Hemoglobin values between 3.1 g % to 5 g %.

Table 4: Range of leukocyte count in patients with pancytopenia

Leukocyte count (cells/cumm)	No. of cases	Percentage
1000-2000	2	7.1
2100-3000	13	46.4
3100-4000	13	46.4
Total	28	100

The total leukocytic count was in the range of 1100-3800 cells/cumm. Most (92.8 %) of the patients had values in the range of 2100-4000 cells/cumm. 7.1 % of the patients had values in between 1000-2000 cells/cumm.

Table 5: Range of platelet count in patients with pancytopenia

Platelet count (cells/cumm)	No. of cases	Percentage
25,000-50,000	1	3.6
51,000-75,000	1	3.6
76,000-1,00,000	12	42.8
1,01,000-1,50,000	14	50
Total	28	100

The range of platelet count varied from 30,000-1,44,000 cells/cumm. Most (50%) patients had platelet counts in the range of 1,01,000-1.50,000 cells/cumm followed by 42.8 % of cases with a platelet count of 76,000-1,00,000 and one case each of having total platelet count between 25,000-50,000 and 51,000-75,000.

Table 6: Bone marrow cellularity in patients with pancytopenia

Leukocyte count (cells/cumm)	No. of cases	Percentage
Hypercellular	17	68
Hypocellular	7	28
Normocellular	1	4
Total	25	100

We have observed that most of the smears were hypercellular (68 %), while 28 % showing hypocellularity and only single case has normocellular bone marrow (4 %).

Table 7: Table showing various causes of pancytopenia

Impression	No. of cases	Percentage
Aplastic anemia	4	14.3
Megaloblastic anemia	9	32.1
ALL	5	17.8
Myelodysplastic syndrome	5	17.8
Hypersplenism	1	3.6
Viral infection	2	7.14
Typhoid	2	7.14
Total	28	100

As evident from the above table that most of the pancytopenia cases belong to megaloblasticanemia (32.1) and followed by 5 cases each of ALL and Myelodysplastic syndrome (17.85 %). We also found that 4 cases of aplastic anemia percent 14.3 with pancytopenia and two cases each of viral infection and typhoid (7.14 %), while only a single case of hypersplenism was diagnosed as a case of pancytopenia.

DISCUSSION

In the study conducted by Tilak and Jain (1999), megaloblasticanemia was identified as the most common cause of pancytopenia, accounting for 68% of cases [13]. Similar findings were observed by Ishtiaq *et al.* (2002) (39%) [14] and Dodhy *et al.* (2005) (35.95%) [15]. Rahim *et al.* (2005) also reported megaloblasticanemia as the primary cause, although in a slightly lower percentage (24.9%) compared to the present study and others [16].

Contrasting results were found in the study by Retief and Heyns (1976), where bone marrow failure was the leading cause (67.7%), followed by aplastic anemia in 52.7% of cases in the international agranulocytosis and aplastic anemia study [17]. Hussain *et al.* and Verma and Dash in 1992 reported a higher incidence of aplastic anemia (40.6%) in cases of pancytopenia [18]. These findings differ from studies conducted by Khodke *et al.* (2002), Kumar *et al.* (2001), and Jha *et al.* (2008), which identified aplastic anemia as the most common cause (29.5%) rather than megaloblasticanemia [19-21].

Imbert *et al.* (1989) reported that malignant myeloid disorder was the primary cause of pancytopenia (42%) in their study, contrasting with aplastic anemia or megaloblasticanemia [22]. Nutritional deficiencies, including iron deficiency in combination with folate or B12 deficiency, were also identified as causes of pancytopenia, resulting in mixed deficiency and macro-and microcytic anemia. In the present study, both myelodysplastic syndrome and acute lymphoblastic leukemia accounted for the second most common cause of pancytopenia (17.8%), which aligns with the study by Imbert *et al.* (1989) that reported 18% cases of malignant lymphoid disorders. Most other studies showed megaloblasticanemia as the second most common cause (Varma and Dash, 1992; Khodke *et al.*, 2002;Jha *et al.*, 2008) [18-21].

Pancytopenia occurring in myelodysplastic syndrome (MDS) is less common compared to mono-and bicytopenia. Greenberg *et al.* (2012) found pancytopenia in 15% of the 816 MDS patients in their study, while the present study of 28 cases identified 5 MDS cases, all with pancytopenia. However, Kini *et al.* (2001) found bicytopenia to be more common than pancytopenia in their study of 31 patients. The present study identified two cases of typhoid and viral fever out of 28 cases (7.2%) as causes of pancytopenia, which aligns with Udden *et al.* (1986) and Sood *et al.* (1997). Hypersplenism, known to cause pancytopenia through blood cell sequestration, accounted for only one case (3.6%) in the present study, similar to the study by Shazia Menon *et al.* (2008) on 230 cases of pancytopenia, which reported a 4.35%incidence of hypersplenism. However, Kumar *et al.* (2001) reported a higher incidence of hypersplenism in 11.5% of cases, and Retief (1976) found hypersplenism as the cause in 7.75% of pancytopenia cases in their study of 195 cases. These results indicate a higher prevalence of hypersplenism compared to the present study.

In summary, the findings of various studies on the causes of pancytopenia demonstrate some variations. Megaloblasticanemia emerged as the most common cause in several studies, including the present study, while others reported aplastic anemia as the primary cause. Malignant myeloid disorders, myelodysplastic syndrome, and acute lymphoblastic leukemia were also identified as significant contributors to pancytopenia in some studies. Nutritional deficiencies, viral infections, typhoid, and hypersplenism were found to play a role in certain cases. The variations in findings could be attributed to factors such as differences in sample sizes, geographical locations, and patient populations studied.

It is crucial to consider these diverse findings when approaching the diagnosis and management of pancytopenia, as they highlight the need for a comprehensive evaluation of patients, including clinical features, hematological parameters, and bone marrow examination. Further research and larger-scale studies are warranted to gain a more comprehensive understanding of the underlying causes and optimal investigative approaches to pancytopenia.

CONCLUSION

This two-year study analyzed 28 cases of pancytopenia in patients attending J. L. N. Medical College and associated hospitals. The main findings were as follows: megaloblasticanemia was the most common cause (32.1%), followed by acute lymphoblastic leukemia (17.8%), myelodysplastic syndrome (17.8%), aplastic anemia (14.3%), viral infection and typhoid (7.14% each), and hypersplenism (3.6%). Male patients were more affected (ratio 1.33:1), and the age groups of 11-20 and 31-40 had the highest number of cases. Megaloblasticanemia was most prevalent in the 31-40 age group (44.4%), while acute lymphoblastic leukemia cases were found in children aged 18 mo to 9 y. Aplastic anemia accounted for 14.3% of cases, and infections (typhoid and viral fever) comprised 7.14% each. Hypersplenism was the least common cause (3.6%).

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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