



RETICULOCYTE PRODUCTION INDEX- A KEY TO ASSESS BONE MARROW RESPONSE IN CASES OF PANCYTOPENIA.

Pathology

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ABSTRACT

Background: The Reticulocyte Production Index (RPI) is a calculated parameter used in hematology to assess bone marrow function and erythropoiesis. RPI offers a significant advantage by helping clinicians distinguish between causes like aplastic anemia, myelodysplastic syndromes, or megaloblastic anemia, thus streamlining diagnostic and therapeutic pathways. **Objectives:** This study aims to evaluate the effectiveness of Reticulocyte Production Index (RPI) in diagnosing and differentiating the underlying causes of pancytopenia. It also helps to avoid invasive procedures like bone marrow aspiration and biopsy. **Methods:** Analysis of 150 cases of pancytopenia was done. Blood samples of the patients were collected in a 3 ml EDTA vial. Reticulocyte Production Index (RPI) was calculated for each patient which helped to differentiate and identify underlying cause of pancytopenia.

Results:

- Age of pancytopenia patients ranged from 5 to 75 years.
- Males (60%) were more frequently affected than females (40%)
- The majority of cases were of megaloblastic anemia (52%) in adults having RPI < 2 followed by malaria (13.3%) and dengue (8.6%) in pediatric age group having RPI > 2.

Conclusion: The Reticulocyte Production Index (RPI) has emerged as a simple yet highly effective diagnostic tool for pancytopenia. Its ability to provide quick and accurate differentiation of pancytopenia causes makes it a practical and cost-effective frontline diagnostic tool. By providing a non-invasive and reliable diagnostic pathway, RPI minimizes the need for invasive procedures like bone marrow aspirations, which significantly enhances patient comfort and reduces resource burden in clinical settings.

KEYWORDS

Reticulocyte production index, Pancytopenia, Erythropoiesis, Bone marrow

INTRODUCTION

Pancytopenia is a hematological condition characterized by a reduction in all three major blood cell lines: red blood cells, white blood cells, and platelets⁽¹⁾. A systematic approach is required to evaluate the etiology of pancytopenia⁽²⁾. It represents a diverse range of underlying pathologies, making its diagnosis both complex and critical for effective management. One of the primary challenges in managing pancytopenia is differentiating its causes, which range from benign nutritional deficiencies to severe bone marrow disorders.

Reticulocyte indices are critical for the diagnosis of many hematologic diseases⁽³⁾. The Reticulocyte Production Index (RPI) is an important diagnostic tool used to evaluate bone marrow function and erythropoietic activity. The RPI adjusts for the degree of anemia and provides a more accurate picture of bone marrow activity. This is especially vital in cases of pancytopenia, where the raw reticulocyte count may be misleading due to overlapping causes such as nutritional deficiencies, marrow suppression, or infiltrative disorders. It also emphasizes the role of RPI in identifying marrow activity in cases of pancytopenia⁽⁴⁾. It also highlights RPI's utility in distinguishing various underlying causes of pancytopenia⁽⁵⁾.

Inclusion Criteria

- **Hemoglobin (Hb) Thresholds⁽⁶⁾:**
Males: Hb < 13.5 g/dL
Females: Hb < 11.5 g/dL
- **Total Leukocyte Count (TLC):**
TLC < 4,000 cells/cumm
- **Platelet Count:**
Platelet count < 1.5 lakh/cumm
- Patients presenting with pancytopenia based on these criteria were included, ensuring a focus on clinically significant cases.

Procedure

Sample Collection

- Blood samples were collected in 3 mL EDTA vacuttes to maintain stability and prevent coagulation during analysis.
- Peripheral blood smears were prepared, and complete blood counts (CBCs) were performed using a 5 part automated hematology analyser.

- To stain reticulocytes, we used methylene blue stain (supravital stain) and reticulocytes appeared blue under the microscope.

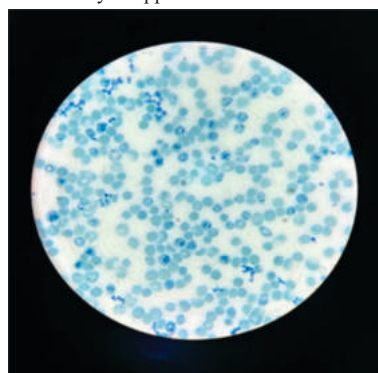


Figure 1: Stained Reticulocytes Under Microscope

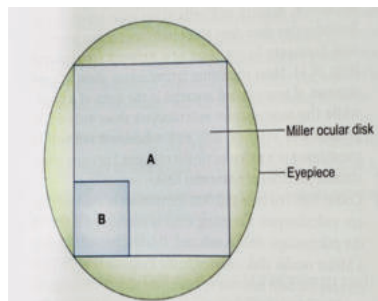


Figure 2: Miller Ocular Disk for Counting Reticulocytes

Miller ocular disk is used to facilitate counting of reticulocytes⁽⁷⁾

It has two squares- A and B

Square A is 9 times larger than square B

Square A is used for counting reticulocytes while square B is used for counting RBC.

Reticulocyte Count- Total Reticulocytes Counted in Square A x 100 Total RBC Counted in Square B x 9

Corrected Reticulocyte Count: Adjusted based on the degree of anemia.

$$\frac{\text{Reticulocyte (\%)} \times \text{Patient's Hematocrit}}{\text{Normal Hematocrit (45\%)}}$$

A value of 45% is usually used as a normal hematocrit for males.

RPI Calculation⁽⁸⁾: Adjusted further for reticulocyte maturation time (based on the hematocrit level).

$$\frac{\text{Corrected Reticulocyte Count}}{\text{Maturation Time in Peripheral Blood in Days}}$$

Hematocrit (Hct) Level	Maturation Correction Factor in blood
36%-45%	1.0
26%-35%	1.5
16%-25%	2.0
<15%	2.5

Table 1 : Maturation Stages of Reticulocyte

Maturation stage	Morphological description
STAGE 0	Orthochromatic normoblast (nrbc)
STAGE I	Dense cohesive reticulum in nrbc
STAGE II	Extensive network of loose reticulum
STAGE III	Small reticulum with scattered granules
STAGE IV	Scattered granules

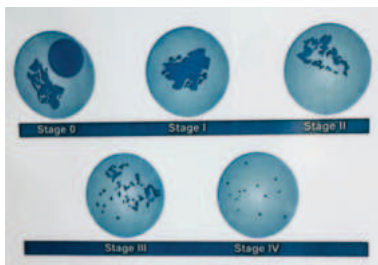


Figure 3 : Methylene Blue Stain Illustrating Reticulocyte Stages.

RESULTS

In the present study, out of 150 patients 90 (60%) patients were male and 60 (40%) were females.

Male and female ratio is 1.5:1 suggesting males are more affected than females.

RPI values were analyzed to determine bone marrow activity:

- **RPI > 2 (Hyperproliferative Bone Marrow):** Suggests appropriate marrow response (e.g., hemolytic anemia).
- **RPI < 2 (Hypoproliferative Bone Marrow):** Indicates inadequate marrow response (e.g., aplastic anemia or megaloblastic anemia).



Figure 4 : Interpretation of Reticulocyte Production Index.

Table 2 : Gender wise incidence of pancytopenia		
Gender	No. of patients	Percentage (%)
Male	90	60
Female	60	40
Total	150	100

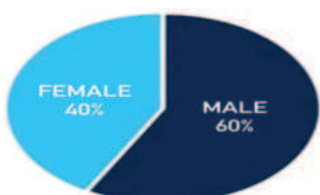


Chart 1: Incidence of Pancytopenia

Table 3 : Distribution of causes of pancytopenia

Etiology	Number of cases of pancytopenia	Percentage (%)
Megaloblastic anemia	78	52%
Malaria	20	13.3%
Dengue	13	8.7%
Aplastic anemia	11	7.3%
Myelodysplastic syndrome	10	6.7%
Leukemia	7	4.7%
Autoimmune hemolytic anemia	3	2.0%
Thalassemia	2	1.3%
Others	6	4.0%
Total	150	100.0%

Table 4: Mean RPI for various etiology

Etiology	Mean RPI
Megaloblastic anemia	0.85
Malaria	2.1
Dengue	2.3
Aplastic anemia	0.4
Myelodysplastic syndrome	1.5
Leukemia	1.1
Autoimmune hemolytic anemia	2.0
Thalassemia	1.8
Others	1.2

DISCUSSION

This study highlights the significant role of the Reticulocyte Production Index (RPI) in diagnosing and differentiating the causes of pancytopenia in the Indian context. The findings reveal megaloblastic anemia as the most common etiology. There is widespread impact of nutritional deficiencies, particularly vitamin B12 and folate, across India which is more common in males due to chronic alcoholism.

Additionally, infectious causes, including malaria (13.3%) and dengue (8.7%) in pediatric patients were identified as significant contributors. The study also demonstrated distinct RPI values across etiologies, with megaloblastic anemia (0.85), aplastic anemia (0.40), malaria (2.10), and dengue (2.3) showing clear differentiation.

Reticulocyte production index has high sensitivity (88.5%) and specificity (85%) which is supported by studies in Chennai demonstrating its effectiveness in distinguishing hemolytic and non-hemolytic anemia⁽⁹⁾

Furthermore, RPI significantly reduced the need for invasive diagnostics, with 88.6% of cases avoiding bone marrow aspiration, findings from Uttarakhand on minimizing invasive procedures⁽¹⁰⁾

These values reinforce RPI's utility in diagnosing pancytopenia and reducing diagnostic ambiguity. The study emphasizes the importance of integrating RPI into routine diagnostic workflows, which can enable early identification of reversible causes such as nutritional deficiencies and reduce resource utilization by avoiding unnecessary invasive procedures.

Finally, these findings stress the need for public health initiatives to address nutritional deficiencies and endemic infections, which remain major drivers of pancytopenia in India. By leveraging RPI, clinicians can significantly enhance patient outcomes and improve resource efficiency in the Indian healthcare system.

CONCLUSION

The Reticulocyte Production Index (RPI) has emerged as a simple yet highly effective diagnostic tool for pancytopenia. It aids in the early differentiation of underlying causes, particularly distinguishing nutritional anemias from other etiologies such as infections and bone marrow failure.

Its ability to provide quick and accurate differentiation of pancytopenia causes makes it a practical and cost-effective frontline diagnostic tool. The integration of RPI into routine hematology workflows can streamline diagnostics, ensuring timely interventions and better patient outcomes, especially in areas with a high prevalence of reversible causes like nutritional deficiencies.

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