

# Utility of reticulocyte indices in the diagnosis of pancytopenia

Jonali Das<sup>1</sup>, Yookarin Khonglah<sup>1</sup>, Iadarilang Tiewsoh<sup>2</sup>,  
Zachariah Chowdhury<sup>1</sup>, Himesh Barman<sup>3</sup>

Departments of <sup>1</sup>Pathology, <sup>2</sup>Medicine and <sup>3</sup>Pediatrics, NEIGRIHMS, Shillong, Meghalaya, India

## ABSTRACT

**Introduction:** Pancytopenia is a hematological condition in which there is a reduction in all three cell lines of blood. This study aims to evaluate the utility of reticulocyte indices such as reticulocyte % (retic %), immature reticulocyte fraction (IRF), and mean reticulocyte volume (MRV) in identifying the cause of pancytopenia. **Materials and Methods:** Reticulocyte indices were measured by an automated coulter. These values were then correlated with relevant biochemical and bone marrow results and cases were stratified into different etiological groups. Receiver operator curve (ROC) analysis was performed and various cut-off values were derived based on the reticulocyte indices. ROC was repeated to further classify cut-off values at every level to help formulate a diagnostic algorithm. **Results:** A total of 154 cases of pancytopenia were obtained. Ages ranged from 7 months to 87 years with a mean of 42, the male:female ratio was 1.08:1. The majority of the cases were megaloblastic anemias in which the cut-off values for retic % was <0.91 with a sensitivity of 78.1% and specificity of 70%, IRF was 0.45 with a sensitivity of 76.7% and specificity of 64%, and MRV was >121.8 fl with a sensitivity of 83.6% and specificity of 80%. The values on ROC could segregate nutritional from nonnutritional causes. The IRF and MRV also helped to differentiate megaloblastic anemia from dual deficiency anemia. **Conclusion:** Reticulocyte indices help identify the cause of pancytopenia. They can segregate nutritional anemia from other causes of pancytopenia allowing presumptive treatment to be initiated and may obviate invasive procedures such as bone marrow examination.

**Keywords:** IRF, MRV, pancytopenia, reticulocyte percentage

## Introduction

Pancytopenia is a hematological condition in which there is a reduction in all three cell lines of blood, i.e., red blood cells, white blood cells, and platelets.<sup>[1]</sup> It is not by itself a disease but results from a number of disease processes that primarily or secondarily involve the bone marrow. The underlying pathology determines the management and prognosis of the patient.

A systematic approach is required to evaluate the etiology of pancytopenia.<sup>[2]</sup> Reticulocyte indices are critical for the diagnosis of many hematologic diseases.<sup>[3]</sup> They play a vital role in the monitoring of patients receiving conventional or experimental therapies for various diseases.

Hematology analyzers can count reticulocytes and quantify their maturity. Automated evaluation helps in providing accurate information about the age distribution of the reticulocyte population, eliminates any subjective technical variability and imprecise counting of manual evaluation, and is cost-efficient for the evaluation of a large number of samples.<sup>[3,4]</sup> Nowadays, newer reticulocyte indices like reticulocyte count and percentage, mean reticulocyte volume (MRV), and immature reticulocyte

**Address for correspondence:** Dr. Yookarin Khonglah,  
Department of Pathology, NEIGRIHMS, Shillong - 793 018,  
Meghalaya, India.  
E-mail: yookarink@gmail.com

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fraction (IRF) by automated hematology analyzers help us to get an insight into the marrow erythropoietic activity and are useful in the evaluation of pancytopenia.

Reticulocyte percentage (retic %) indicates the rapidity of red blood cell turnover. Normal retic % is 1%–2% and represents the daily replacement of 0.8%–1% of the circulating red blood cells.<sup>[2]</sup>

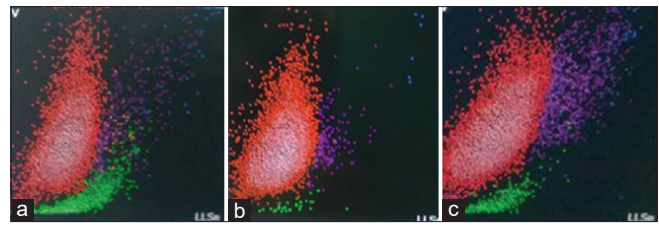
The term IRF was proposed to indicate the least mature fraction of reticulocytes.<sup>[5]</sup> It is the ratio of immature reticulocytes to the total number of reticulocytes. This parameter is an early and sensitive index of erythropoietic activity of the bone marrow. The best clinical use, in the classification of anemias based on the evaluation of erythropoietic response, can be obtained.<sup>[6]</sup> The IRF result is reported along with the reticulocyte count (both percentage and absolute value). Its reference value is 0.11–0.38.<sup>[7]</sup>

MRV is 24% higher than the mean cell volume (MCV) of mature erythrocytes. The MRV multiplied by the number of reticulocytes gives the “hematocrit” value of the reticulocytes. It helps in the monitoring of treatment response and also is a sensitive marker of erythropoiesis.<sup>[8]</sup>

So far, megaloblastic anemias and dual deficiency anemias (DDA) are diagnosed by bone marrow aspiration which is invasive, or by biochemical parameters which are costly, and which may not be reliable since values are vulnerable to change with a good diet in the hospital.<sup>[9]</sup> Reticulocyte indices can play a pivotal role in identifying the cause of pancytopenia avoiding invasive procedures or expensive testing. Hence, this study is done to evaluate the significance of reticulocyte indices as deciding parameters in identifying and differentiating the various causes of pancytopenia and this is depicted in a diagnostic algorithm. This diagnostic algorithm may be utilized by primary care physicians for rapid diagnosis and management of pancytopenia in correlation with the clinical presentation.

## Materials and Methods

This is a hospital-based prospective analytical study, carried out over a period of 2 years (2019–2020). Consent was taken from all the patients. The identity of the patients was not revealed and data collected were entered in a de-identified format. Blood samples were collected in a 3 ml EDTA vial and were processed in the Beckman coulter DxH 800. Samples with values of Hb <13.5 g/dl in males and <11.5 g/dl in females, TLC <4000/cumm, and platelet count <1.5 lakhs/cumm were included in the study. Chemo/radiotherapy induced pancytopenia and patients who have received blood transfusion were excluded. Reticulocyte indices were studied on both patient and control samples [Figure 1] and correlated with biochemical and bone marrow findings. A diagnostic algorithm for the etiological classification of pancytopenia based on the receiver operative characteristics (ROC) curve analysis of reticulocyte indices was then formulated.



**Figure 1:** Scatter graphics. (a) Reticulocyte indices in a case of megaloblastic anemia (low reticulocyte %, mildly raised IRF, and high MRV). (b) Reticulocyte indices in a case of chronic kidney disease (low reticulocyte%, low IRF, and normal MRV). (c) Reticulocyte indices in a case of myelodysplastic syndrome (normal reticulocyte %, mildly raised IRF, and higher MRV)

## Ethical committee approval and informed consent

The study was approved by the Institutes Ethics Committee (NEIGR/IEC/M7/T11/19).

## Statistical analysis

Statistical package for social sciences (SPSS), IBM SPSS Statistics 20.0 was used for statistical analysis. Mean, median, standard deviation, percentage, 95% confidence interval, independent sample t-test, and ROC curve analysis were used and  $P < 0.05$  was considered statistically significant.

## Results

Out of the 154 cases, 80 (51.94%) were males while 74 (48.05%) were female patients, with a male to female ratio of 1.08:1. The patient's age varied from 7 months to 87 years with a mean age of 42 years. The most common cause of pancytopenia in males and females was megaloblastic anemia. The other underlying causes of pancytopenia are tabulated in Table 1.

There existed a significant difference ( $P < 0.001$ ) in the reticulocyte indices between megaloblastic anemia with control and DDA with control. We also found a significant difference ( $P < 0.001$ ) in the IRF and MRV value of megaloblastic anemia versus DDA, but there was no difference ( $P = 0.238$ ) in the value of retic % of megaloblastic anemia versus DDA [Table 2].

On ROC curve analysis, cut-off values of <0.91 for retic % with sensitivity of 78.1% and specificity of 70%, >0.45 for IRF with a sensitivity of 76.7% and specificity of 64% and >121.8 fl for MRV with a sensitivity of 83.6% and specificity of 80% were found in megaloblastic anemia. These values helped in differentiating megaloblastic anemia cases from healthy controls [Figure 2].

Cut-off values of >0.76 for retic % with sensitivity of 83.6% and specificity of 80%, >0.42 for IRF with a sensitivity of 54.8% and specificity of 66.7%, and <102.3 fl for MRV with a sensitivity of 86.3% and specificity of 83.3% were found in DDA. These values help in differentiating DDA from healthy controls [Figure 3].

Cut-off values of >0.76 for retic % with sensitivity of 58.3% and specificity of 80%, >0.44 for IRF with a sensitivity of 86.7%

and specificity of 73.3%, and >111.8 fl for MRV with a sensitivity of 96.7% and specificity of 93.3% helped in differentiating megaloblastic anemia from DDA [Figure 4].

A diagnostic algorithm for evaluating the cause of pancytopenia based on ROC curve analysis of reticulocyte indices was formulated and illustrated in Figure 5.

### Discussion

Pancytopenia is a hematological condition in which there is a reduction in all three cell lines of blood.<sup>[10]</sup> This study aims to analyze the utility of reticulocyte indices such as retic %, IRF, and MRV in formulating a diagnostic algorithm for identifying the etiology of pancytopenia.

In our study, megaloblastic anemia was the most common cause of pancytopenia. Concurrent findings were seen in other studies conducted in India.<sup>[9,10]</sup> This reflects the higher prevalence of nutritional anemia in the Indian population.<sup>[11]</sup>

The retic % was decreased in both megaloblastic anemia and DDA compared to healthy controls. A statistically significant difference ( $P < 0.001$ ) in retic % value between control with

megaloblastic anemia and control with DDA was found. The retic % could not differentiate between megaloblastic anemia and DDA ( $P = 0.238$ ) similar to the study conducted by Priya et al.,<sup>[2]</sup> Sindhu et al.,<sup>[12]</sup> and Balci et al.<sup>[13]</sup> However, Gomez et al.<sup>[14]</sup> in their study found the mean retic % as 1.5% which was higher than their normal control (1.4%), and suggested that this could be due to the post-therapy effect.

In our study, high retic % was seen in hematological neoplastic conditions, cases of blood loss, and peripheral destruction of RBCs. Similar results were seen in a study done by Sindhu et al.<sup>[12]</sup> where hypersplenism, acute blood loss, and neoplastic conditions like aleukemic leukemia had higher retic % compared to other causes of pancytopenia. Priya et al.<sup>[2]</sup> found that mean retic % was higher in malaria, sepsis, metastasis, lymphoid neoplasms, and leukemia. In their study, the highest retic % was seen in malaria and sepsis. Our study did not include cases of malaria or sepsis and the highest retic % was seen in hematological neoplastic conditions.

The IRF is an early and sensitive index of erythropoiesis; in fact, immature reticulocytes emerge in a greater proportion as the red cell production increases. It is a useful parameter to evaluate the erythropoietic activity and the clinical utility is greatest in the classification of anemia based on marrow response.<sup>[15]</sup> In the present study, IRF was analyzed in all cases of pancytopenia. It was found that IRF value was highest in cases of hematological neoplastic conditions and cases with peripheral destruction of blood cells (acute blood loss and hypersplenism) compared to nutritional anemia, chronic diseases, and aplastic anemia.

Ray et al.<sup>[16]</sup> also found the highest IRF value in cases of malignancy, hypersplenism, and autoimmune diseases which correlated with our study, and IRF value was only marginally high in megaloblastic anemia and myelodysplastic syndrome (MDS).

In the present study, a statistically significant difference ( $P < 0.001$ ) was found for IRF value between control and megaloblastic anemia; control and DDA; and megaloblastic anemia with DDA.

Gomez et al.<sup>[14]</sup> and Kim et al.<sup>[17]</sup> reported that the value of IRF for megaloblastic anemia (0.38) was higher than the healthy control group (0.24), which was statistically significant with a  $P$  value of  $< 0.01$ . Similar findings were also noted in our study.

We had 24 cases of pancytopenia due to chronic disease conditions and all the cases showed a lower IRF value with a cut-off value of  $< 0.32$  derived by ROC curve analysis. These

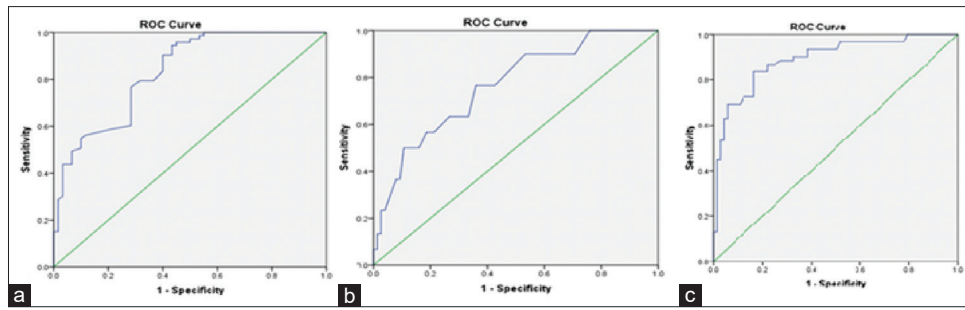
**Table 1: Etiological profile of pancytopenia**

Etiology	Number of cases	Percentage
Megaloblastic anemia (MA)	60	38.96
Dual deficiency anemia (DDA)	30	19.48
Chronic kidney disease (CKD)	11	7.14
Chronic liver disease (CLD)	8	5.19
Myelodysplastic syndrome (MDS)	6	3.89
Acute leukemia (AL)	5	3.24
Aplastic anemia (AA)	5	3.24
Lymphoma (LYM)	5	3.24
Plasma cell dyscrasia (PCD)	4	2.59
Hemophagocytic syndrome (HPS)	3	1.94
Rheumatoid arthritis (RA)	3	1.94
Immune thrombocytopenia (ITP)	3	1.94
Tuberculosis (TB)	2	1.29
Autoimmune hemolytic anemia (AIHA)	2	1.29
Hypersplenism (HS)	2	1.29
Systemic lupus erythematosus (SLE)	2	1.29
Dengue hemorrhagic fever (DEN)	1	0.65
Pure red cell aplasia (PRCA)	1	0.65
Thalassemia (THAL)	1	0.65
Total	154	100

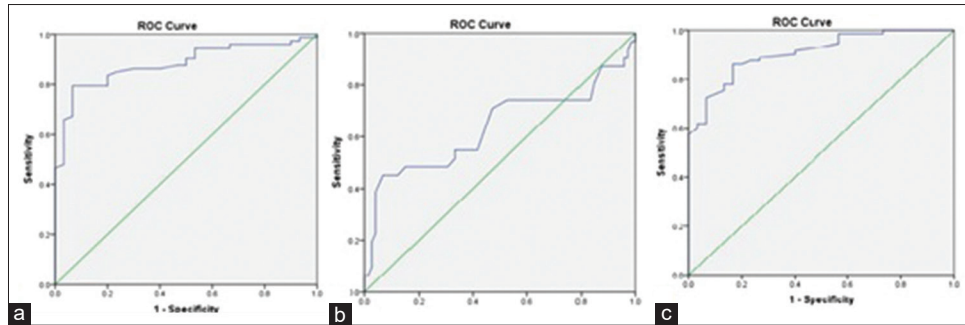
**Table 2: Reticulocyte indices of control, megaloblastic anemia, and dual deficiency anemia**

	Control (Group 1)	MA (Group 2)	DDA (Group 3)	P		
				Group 1-2	Group 1-3	Group 2-3
Reticulocyte %	1.27±0.29	0.81±0.07	0.6±0.26	<0.001	<0.001	0.238
IRF	0.42±0.09	0.52±0.12	0.47±0.16	<0.001	<0.001	<0.001
MRV	114.2±11.83	139±17.06	98.35±7.89	<0.001	<0.001	<0.001

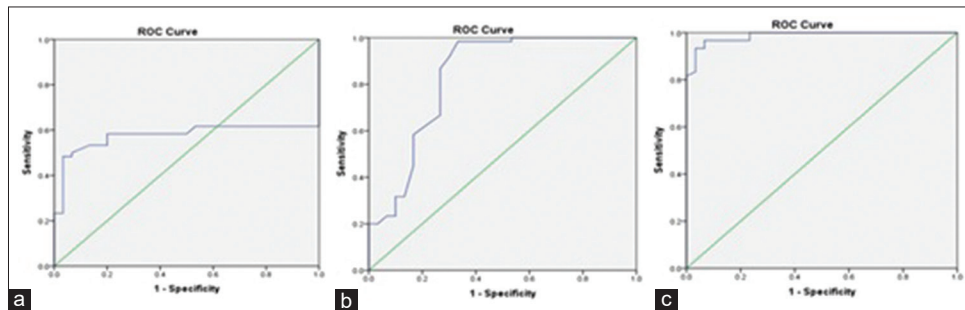
MA - megaloblastic anemia, DDA - dual deficiency anemia, IRF - immature reticulocyte fraction, MRV - mean reticulocyte volume



**Figure 2:** ROC curve for reticulocyte indices of megaloblastic anemia: (a) ROC curve for reticulocyte % of megaloblastic anemia compared to control. (b) ROC curve for IRF of megaloblastic anemia compared to control. (c) ROC curve for MRV of megaloblastic anemia



**Figure 3:** ROC curve for reticulocyte indices of dual deficiency anemia. (a) ROC curve for reticulocyte % of dual deficiency anemia compared to control. (b) ROC curve for IRF of dual deficiency anemia compared to control. (c) ROC curve for MRV of dual deficiency anemia compared to control



**Figure 4:** ROC curve for reticulocyte indices of megaloblastic anemia compared to dual deficiency anemia: (a) ROC curve for reticulocyte %. (b) ROC curve for IRF. (c) ROC curve for MRV

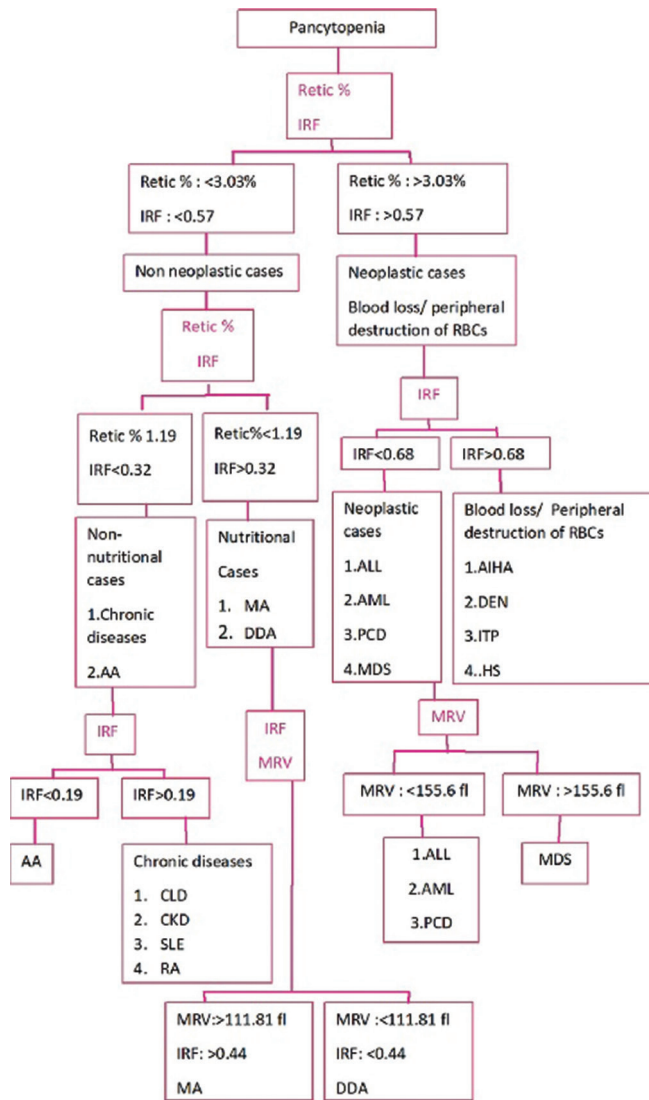
chronic diseases such as chronic kidney disease (CKD), chronic liver disease (CLD), systemic lupus erythematosus (SLE), and rheumatoid arthritis lead to decreased erythropoiesis, and the bone marrow is nonresponsive or under-responsive to anemia. Our findings were similar to a study done by Chang *et al.*<sup>[18]</sup>

In the present study, MRV value was increased in cases of megaloblastic anemia, MDS, CLDs and decreased in DDA cases. There was a statistically significant difference in MRV between controls and megaloblastic anemia; controls and DDA; and between megaloblastic anemia and DDA. Balci *et al.*<sup>[13]</sup> reported the mean MRV of megaloblastic anemia to be  $107.14 \pm 5.71$  fl. Kim *et al.* found the mean MRV of megaloblastic anemia to be 127.07 fl., which was more than the control MRV value; this is similar to our finding.<sup>[17]</sup> In the study done by Gomez *et al.*,<sup>[14]</sup> a similar finding of the MRV value being higher in megaloblastic anemia than that of the control group was noted.

In the current study, the highest MRV value was seen in all 6 cases of MDS. Gomez *et al.*<sup>[14]</sup> and Kim *et al.*<sup>[17]</sup> also reported the highest MRV value in all their cases of MDS.

Gomez *et al.*<sup>[14]</sup> had concluded that this circulating, highly immature reticulocyte could represent dyserythropoiesis with the persistence of abnormal cytoplasmic structures, and elevated amounts of RNA in dysplastic clones. Hence, the measurement of reticulocyte maturation parameters like IRF and MRV may be very useful tools in the differential diagnosis of macrocytic anemia. The MRV value may be especially useful to differentiate megaloblastic anemia with trilineage dyspoiesis from cases of MDS.

We derived various cut-off values for pancytopenia cases, based on the reticulocyte indices by using ROC curve analysis. The ROC curve analysis was repeated to further classify cut-off



**Figure 5:** Diagnostic algorithm for pancytopenia cases based on cutoff value of reticulocyte indices. IRF, immature reticulocyte fraction; MA, megaloblastic anemia; DDA, dual deficiency anemia; CKD, chronic kidney disease; CLD, chronic liver disease; SLE, systemic lupus erythematosus; AA, aplastic anemia; AL, acute leukemia; LYM, lymphoma; PCD, plasma cell dyscrasia; AIHA, autoimmune hemolytic anemia; DEN, dengue hemorrhagic fever; ITP, immune thrombocytopenic purpura; HS, hypersplenism; MDS, myelodysplastic syndrome

values at every level to help form a diagnostic algorithm as indicated [Figure 5].

The ROC curve analysis was done for retic % and a cut-off value of 3.03 with a sensitivity of 92.3% and specificity of 91.6% was derived, which could be used as a screening test in pancytopenia to differentiate between neoplastic and nonneoplastic causes. Retic % of >3.03% was considered as neoplastic and <3.035% as nonneoplastic. Further, a cut-off value of >1.19% with a sensitivity of 78.3% and specificity of 81.1% was found to differentiate between nonnutritional and nutritional causes in nonneoplastic conditions. Nonnutritional causes had a cut-off value of >1.19%. Therefore, these values may allow presumptive

treatment to be initiated in nutritional anemias avoiding invasive procedures such as bone marrow examination for diagnosis.

The ROC curve analysis for IRF revealed a cut-off value of >0.57 with a sensitivity of 95% and specificity of 83.5%. The hematologic neoplastic conditions and cases of blood loss had higher IRF values of >0.57. The further classification was done among cases with an IRF value of >0.57 and a cut-off value of 0.68 with a sensitivity of 83.3% and specificity of 92.9% was derived. This value helped to differentiate autoimmune hemolytic anemia, dengue hemorrhagic fever, immune thrombocytopenia, and hypersplenism which had values of >0.68 from neoplastic conditions like acute leukemia, lymphoma, and plasma cell dyscrasia which had values of <0.68. The cases with an IRF value of <0.57 were further classified. Megaloblastic anemia and DDA showed values of >0.32 with sensitivity of 94.65% and specificity of 87% differentiating them from CKD, CLD, SLE, and aplastic anemia which had values <0.32. Again, the cases with values of <0.32 were re-classified and a cut-off of 0.19 of sensitivity of 94.4% and specificity of 100% was derived. This allowed differentiation of CKD, CLD, RA, and SLE with an IRF value of >0.19 from aplastic anemia which had an IRF value of <0.19.

The ROC curve analysis for MRV revealed a cut-off value of >115.75 fl with sensitivity of 94.7% and specificity of 81% which differentiated the macrocytic anemias (megaloblastic anemia, MDS, and CLD) from other causes of pancytopenia. On further classifying, MDS had a cut-off value of >155.6 fl with a sensitivity of 66.7% and specificity of 81.2% which differentiated it from megaloblastic anemia and CLD. Again, megaloblastic anemia had a cut-off value of >123.81 fl with sensitivity of 81.7% and specificity of 66.7%, which differentiated it from CLD cases.

On literature search for the diagnostic utility of reticulocyte indices in pancytopenia for their cut-off values, no relevant matches were found using internet search engines like Google, Yahoo, Bing, and AOL, with keywords such as ROC curve analysis, reticulocyte indices, retic %, IRF, MRV, and pancytopenia in various combinations.

## Conclusion

In summary, these cut-off values of reticulocyte indices enabled us in identifying the causes of pancytopenia and in construction of a diagnostic algorithm. This algorithm will especially help the primary care physicians in early diagnosis and management of nutritional causes of pancytopenia.

## Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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