

Study of Absolute Reticulocyte Count in Evaluation of Pancytopenia

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Abstract

Original Research Article

Background: Pancytopenia is usually due to a problem with the bone marrow that produces the blood cells. However, there can be several different underlying causes. Bone marrow biopsy is indicated in patients of pancytopenia in which cause is not obvious. Non-invasive methods e.g. absolute reticulocyte count (ARC) may help in preliminary diagnosis to evaluate the cause of pancytopenia. **Objective:** The aim of the study is to evaluate the role of Absolute Reticulocyte Count in evaluation of Pancytopenia. **Methodology:** This prospective study includes 450 patients of pancytopenia who have visited our hospital from June 2012 to June 2013. Patients who satisfied the criteria for pancytopenia were evaluated with clinical details. Pancytopenia work-up including Peripheral blood picture, Complete blood counts and Reticulocyte count. Absolute Reticulocyte Count (ARC) was calculated. **Results:** Among various causes of pancytopenia, megaloblastic anemia is found to be commonest among them. Our study shows various causes of pancytopenia such as megaloblastic anemia (36.66%), Mixed anemia (Dimorphic anemia) (21.11%), aplastic anemia (16.66%), leukemia (3.77%), metastasis (2.7%) and myelodysplastic syndrome (1.11%) Absolute reticulocyte count (ARC) was measured: Aplastic anemia (<25x10⁹/L), myelodysplastic syndrome (25–50x10⁹/L), megaloblastic anemia (50–75x10⁹/L), leukemia (76–100x10⁹/L). **Conclusion:** The present study concludes that Absolute reticulocyte count is an important indicator for determining the cause of pancytopenia and should be done along with peripheral smear in the very beginning. It is also useful in planning further investigations and management.

Keywords: pancytopenia, Absolute reticulocyte count (ARC), Bone marrow.

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INTRODUCTION

Pancytopenia is simultaneous presence of anemia, leukopenia and thrombocytopenia [1, 2]. It is generally due to decrease in hematopoietic cell production in the marrow resulting from infections, toxins, malignant cell infiltration, chemotherapies and radiation [3]. Different studies done at different places showed variable frequency of pancytopenia [4]. Identification of the disease is of prime importance, since this is the key to appropriate management.

Bone marrow examination is extremely helpful in evaluation of Pancytopenia [5, 6]. This allows complete assessment of marrow architecture and the pattern of distribution of any abnormal infiltrate and for the detection of focal bone marrow lesions [7, 8]. While bone marrow failure syndromes and malignancies are important causes, certain non-malignant conditions such as infection and nutritional anemia are equally important causes [6]. The most common causes leading to Pancytopenia on Bone Marrow examination are Hypoplastic (AA) bone marrow (29.05%),

Megaloblastic anemia (MA) (23.64%), Hematological malignancies i.e. Acute Myeloid Leukemia (AML) (21.62%), and Erythroid hyperplasia (EH) (19.6%)

Bone marrow aspiration is considered of primary importance in evaluating the cases of Pancytopenia [9]. Absolute reticulocyte count (ARC) is a calculated index derived from the product of two parameters namely Reticulocyte count percentage and RBC count [10]. It is a marker of red cell production and helps in distinguishing hypo and hyper proliferative anaemias. The normal reticulocyte count is 0.5-2.5% and the normal absolute reticulocyte count is 50-100x10⁹/L. ARC may be very helpful in initial evaluation of pancytopenia but its utility value is often underestimated. Hence in our study we calculated the ARC of patients with pancytopenia, formed an algorithm based on these counts and classified them.

MATERIAL AND METHODS

This prospective case control study was conducted by the Department of Pathology, Govt

Medical college, Bhavnagar and attached hospital, Gujarat, India from June 2012 to June 2013

Study Design

Study includes total 450 patients attending to the Department of General Medicine of the our hospital, who satisfied the criteria for pancytopenia with hemoglobin <9g/dl, WBC<4000/ μ l, Platelets <1,00,000/ μ l [11] were included in our study.

After obtaining complete history of the patients, 5 ml blood samples was collected from all participants and transferred in to EDTA and plain vaccutainer. An Uniq ID was given to each participants to hide the identity of the patients.

All the patients were subjected to examination of their complete blood count, reticulocyte count (RC) and peripheral blood smear. Bone marrow aspiration and/or bone marrow biopsy was done in cases where required. Marrow were examined for cellularity, myeloid: Erythroid ratio, erythropoiesis, myelopoiesis, megakaryopoiesis, other cells such as plasma cells, lymphocytes, blasts and parasites. Special investigations were done in cases where indicated. Patients were followed until discharge and response to therapy was noted.

Plain sample was kept preserved for future investigation like S. vit b12, S.ferritin and folic acid if required.

$ARC \text{ (thousand}/\mu\text{L)} = \text{Reticulocyte \%} \times \text{RBC count (million}/\mu\text{L)} \times 10$.

All cases were compared for diagnosis based on bone marrow biopsy and absolute reticulocyte count.

All data were analyzed statistically by using Microsoft office system to calculate the Mean and SD.

Exclusion Criteria

Diagnosed cases of malignancy, including leukemia receiving chemotherapy or radiotherapy. Patients were selected according to the guidelines of inclusion criteria.

RESULTS

This study includes total 450 patients of pancytopenia. Among them 337(75%) was male and 113(25%) was female participants. The incidence of pancytopenia showed preponderance among males.

Age group of Male patients was 32.50 ± 10.50 while that of female patients was 35.13 ± 16.50 (Table-1).

Table-1: Age and Sex wise distribution of participants (n=450)

Sex	Number	Age Group(yr)
Male	337(75%)	32.50 ± 10.50
female	113(25%)	35.13 ± 16.50
Total	450(100%)	

Table-2: Showing aetiology of pancytopenia

No	Aetiology	No of cases (n)	No of cases (%)
1	Lymphoid neoplasm	14	3.11
2	Aplastic anemia	75	16.66
3	Chronic Malaria	24	5.33
4	Sepsis	45	10
5	Leukemia	17	3.77
6	Megaloblastic anemia	165	36.66
7	Myelodysplastic syndrome	5	1.11
8	Mixed anemia (Dimorphic anemia)	95	21.11
9	Metastasis	10	2.22
	Total	450	100

Among various causes of pancytopenia, megaloblastic anemia is found to be commonest among them. Our study shows various causes of pancytopenia such as megaloblastic anemia (36.66%), Mixed anemia

(21.11%), aplastic anemia (16.66%), leukemia (3.77%), metastasis (2.7%) and myelodysplastic syndrome (1.11%) (Table-2).

Table-3: Hematological finding of pancytopenia cases

No	aetiology	No of case	Hb(g/dl) Mean \pm SD	RBC Mean \pm SD	RDW (%) Mean \pm SD	MCV(fl) Mean \pm SD
1	Lymphoid neoplasm	14	7.35 ± 0.58	2.90 ± 0.25	11.95 ± 0.81	87.05 ± 4.0
2	Aplastic anemia	75	4.50 ± 1.25	2.20 ± 0.15	13.50 ± 1.05	85.25 ± 4.55
3	Chronic Malaria	24	7.40 ± 0.88	4.09 ± 0.39	11.69 ± 0.90	86.50 ± 5.02
4	Sepsis	45	6.88 ± 0.80	3.50 ± 0.35	13.90 ± 1.50	86.64 ± 3.11
5	Leukemia	17	7.05 ± 1.20	3.0 ± 0.27	12.45 ± 0.58	88.90 ± 5.0
6	Megaloblastic anemia	165	5.90 ± 1.10	2.0 ± 0.35	17.50 ± 1.90	118.50 ± 5.50
7	Myelodysplastic syndrome	5	7.9 ± 0.50	3.20 ± 0.25	13.50 ± 0.71	85.50 ± 5.50
8	Mixed anemia (Dimorphic anemia)	95	6.0 ± 1.12	3.10 ± 0.45	15.0 ± 1.38	86.50 ± 6.50
9	Metastasis	10	6.25 ± 0.81	3.30 ± 0.85	10.40 ± 1.07	86.10 ± 3.92
		450				

Table-3 showing hematological finding of pancytopenia causes. A very high MCV of 118.50 ± 5.50 fl is seen in all cases of Megaloblastic anaemias which could be used as an adjunct in the diagnosis. RDW was

found to be higher in nutritional anemias which could be explained by marked anisocytosis as expected in these conditions.

Table-4: showing different clinical manifestation of pancytopenia patients

Clinical features	No of cases (%)
Generalised weakness	441(98)
Anaemia	441(98)
Lymphadenopathy	54(12)
Fever	225(50)
Abdominal pain	202(45)
Jaundice	45(10)
Shortness of breath	383(85)
Mouth ulcer	112(25)
Pedal edema	45(10)
Pruritic spot	171(38)
Bleeding	225(50)
Ascites	23(5)
Bone pain	158(35)

Among clinical features, anemia and generalized weakness were the commonest (98%) for each, followed by shortness of breath (85%), fever (50%), bleeding manifestations such as epistaxis,

hematemesis and malena (50%), abdominal pain (54.5%), bone pain (35%). The least common features were jaundice (10%), pedal edema (10%) were followed by ascites (5%) (Table-4).

Table-5: Reticulocyte count and Absolute Reticulocyte count finding of pancytopenia cases

No	Aetiology	Reticulocyte count (%) Mean± SD	Absolute Reticulocyte count (x10 ⁹ /L Mean± SD)
1	Lymphoid neoplasm	4.10 ±0.10	150.30 ±11.50
2	Aplastic anemia	0.10 ±0.00	20.15 ±2.0
3	Chronic Malaria	6.50 ±1.18	238.85 ±45.50
4	Sepsis	6.10 ±0.65	222.80 ±27.50
5	Leukemia	3.50 ±0.40	138.50± 20.60
6	Megaloblastic anemia	0.10 ±0.00	32.84 ±4.04
7	Myelodysplastic syndrome	0.10 ±0.00	38.50 ±2.05
8	Mixed anemia (Dimorphic anemia)	0.10 ±0.00	34.50 ±1.94
9	Metastasis	4.50 ±0.50	197.10±18.0

ARC was stratified in to $< 50 \times 10^9/L$ (low), $51-99 \times 10^9/L$ (Normal) and $>100 \times 10^9/L$ (High) [12]. All cases of Aplastic anaemia had an ARC of $<25 \times 10^9/L$, which can be taken as an indication for bone marrow aspiration. ARC of $25-50 \times 10^9/L$ has to be evaluated with Serum B12, Folate and Ferritin assays and if these assays are found to be low it rules out the need for a bone marrow aspiration. None of our cases of pancytopenia had a normal ARC of $51-99 \times 10^9/L$. All cases of pancytopenia with high ARC should be evaluated with a Bone marrow aspiration unless there is a history of Sepsis or Malaria. This algorithm stresses the importance of calculating absolute reticulocyte count for all cases of pancytopenia.

DISCUSSION

An accurate reticulocyte count is the key to initial evaluation of pancytopenia. Normally,

reticulocytes are the red cells that have been recently released from the bone marrow. Normal reticulocyte count ranges from 1-2% and reflects the daily replacement of 0.8-1% of the circulating RBC population. Reticulocyte count provides a reliable measure of RBC production. Reticulocyte count and absolute reticulocyte count (ARC) should be done on day one along with CBC in order to avoid therapy related changes in reticulocyte count particularly with nutritional anaemia [13].

Our study clearly demonstrated more risk for pancytopenia in younger peoples with age ranging from 13-47 years. Also prevalence rate among males were 75% and that of females 25.0%. This is quite similar to study conducted in Larkana by Jalbani [14], who also showed 72.5% males and 27.5%.

Studies in Philippines and Nepal [15] reported that males were affected with Aplastic anemia much more frequently than females, which might be a result of higher incidence of occupational exposure to chemicals and of pesticides exposure as a common etiological agent for Aplastic anemia in these countries.

In present study, anemia, generalized weakness and shortness of breath were the most common clinical features in pancytopenic patients comprising of (98%), (98%) and (95%) respectively. Similar results have been reported in studies by Aziz T, Ishtiaq O and Niazi M [16-18].

The findings of our study corresponds with the findings of the study done by Tilak and Jain [19], Khodke *et al.*, [20] and Khunger *et al.*, [21] who in their studies found megaloblastic anemia 68%, 44% and 74% respectively as the most common cause of pancytopenia, followed by aplastic anemia (7.7%), (14%) and (14%).

Although we were unable to determine exact cause of megaloblastic anemia, but vitamin B12 deficiency is more common in adults, while folate deficiency in children. In addition, poor nutrition may be responsible for increased frequency of megaloblastic anemia. However, folate deficiency could be caused by chronic diarrheas and malabsorptive states apart from poor nutrition

ARC was stratified in to $< 50 \times 10^9/L$ (low), $51-99 \times 10^9/L$ (Normal) and $> 100 \times 10^9/L$ (High). All cases of Aplastic anaemia had an ARC of $< 25 \times 10^9/L$, which can be taken as an indication for bone marrow aspiration. ARC of $25-50 \times 10^9/L$ has to be evaluated with Serum B12, Folate and Ferritin assays and if these assays are found to be low it rules out the need for a bone marrow aspiration. None of our cases of pancytopenia had a normal ARC of $51-99 \times 10^9/L$. All cases of pancytopenia with high ARC should be evaluated with a Bone marrow aspiration unless there is a history of Sepsis or Malaria. This algorithm stresses the importance of calculating absolute reticulocyte count for all cases of pancytopenia.

CONCLUSION

The present study concludes that Absolute reticulocyte count is an important indicator for determining the cause of pancytopenia and should be done along with peripheral smear in the very beginning. It is also useful in planning further investigations and management.

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