

Clinicopathological Features of Pancytopenia in Adults and the Role of Bone Marrow Study in Etiological Categorization: A One-Year Cross-sectional Study

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Abstract

Background and Objectives: Pancytopenia is a combination of anemia (with a reduction of the red blood cell count), leukopenia, and thrombocytopenia. Leukopenia is usually mainly due to a reduction in the neutrophil count although the number of other granulocytes, monocytes, and lymphocytes are often also reduced. Pancytopenia is a manifestation of many serious and life-threatening diseases. This study was undertaken to evaluate the clinicopathological features of pancytopenia and the role of bone marrow study for etiological categorization. **Settings and Design:** This is a cross-sectional observational study conducted in a tertiary referral center of north east India, for a period of 1 year (from August 2020 to July 2021). **Materials and Methods:** Proper history taking and clinical examination were done for all patients attending with hemoglobin <10 mg/dL, total leukocyte count <3000 cells/cmm, and platelet count <1 lakh/cmm. The investigations done were peripheral blood smear examination with Leishman stain, special staining, as well as specific biochemical and radiological investigations as per requirement in cases; bone marrow aspiration (under all aseptic precaution); and bone marrow trephine biopsy (under all aseptic precaution). The results are presented in tables and compared with similar studies. **Results:** A total of 42 cases of pancytopenia were evaluated. The age group of 18–20 years was the most commonly affected. Male-to-female ratio was 1.33:1. All the patients presented with generalized weakness and pallor, and a large portion also with abdominal discomfort, hepatomegaly, and splenomegaly. Anisocytosis and macrocytosis were the most common peripheral blood smear findings. Vitamin B12 level was decreased in 75% of all the evaluated cases (15 of 20 cases). Bone marrow was hypercellular in most of the cases (80.95%) followed by hypocellular marrow (14.29%). Megaloblastic erythroid hyperplasia was the most common abnormality found in bone marrow. Megaloblastic anemia was the most common etiology followed by hypoplastic/aplastic anemia. A significant portion (7.14%) also showed bone marrow involvement by myeloma. One case of involvement by metastatic tumor was found. **Conclusions:** Most cases of pancytopenia were due to megaloblastic anemia and so easily curable. The bone marrow study is essential to evaluate a case of pancytopenia to know the exact etiology for proper management.

Keywords: Aplastic, hypoplastic, macrocytosis, megaloblastic, pancytopenia

Key Message: Although megaloblastic anemia is the most common etiology of pancytopenia, some patients of multiple myeloma also present as pancytopenia.

INTRODUCTION

Pancytopenia is not a disease entity by itself but a triad of clinical findings that result from a reduction of all the three cellular components of blood, namely erythrocytes, leukocytes, and platelets leading to anemia, leukopenia, and thrombocytopenia.^[1] Leukopenia is usually due to a reduction in the neutrophil count.^[2] Most of the studies in India used following values for the diagnosis of pancytopenia: hemoglobin <10 g/dL,

total leukocyte count (TLC) <3500/cmm, and platelet count <1 lakh/cmm.^[3,4]

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The etiology of pancytopenia plays a pivotal role in its management. In Indian scenario, it has been observed that megaloblastosis (vitamin B12 and/or folate deficiency) is the commonest cause of pancytopenia. Other important conditions that manifest as pancytopenia are aplastic/hypoplastic anemia, hypersplenism, storage disease, malaria, myelodysplastic syndrome, multiple myeloma, hairy cell leukemia, and others.^[5,6]

The aim and objective of the present study is to evaluate the clinicopathological features and etiological spectrum of pancytopenia with the help of bone marrow aspiration and biopsy.

MATERIALS AND METHODS

This is a cross-sectional observational study conducted in a tertiary referral center for a period of 1 year (from August 2020 to July 2021). All cases of pancytopenia in adults as evidenced by laboratory investigation in both the sexes were included. Patients undergoing radiotherapy/chemotherapy, uncooperative, unwilling patients, and those who do not give consent were excluded. Patients' clinical history and examination findings were recorded from the medical record.

Complete blood count was done in Sysmex XN-550 automated hematology analyzer and correlated with manual method when indicated. Peripheral smears were stained with Leishman stain. Vitamin B12 and folic acid estimations were done when necessary. Bone marrow aspiration was done from the posterior superior iliac spine using disposable Salah needle as per the standard operating procedure. Smears were stained with May Grunwald Giemsa stain. Bone marrow biopsy was performed in cases with hypocellular aspiration. Bone marrow biopsy was done with disposable Jamshidi needle (10 and 11G size with a length of 4.5 inches and a bore diameter of 3 mm and 3.5 mm) using the standard operating procedure, fixed in ethylenediamine tetraacetic acid (EDTA), processed in histopathology department and stained with hematoxylin and eosin for examination.^[7] Bone marrow smears and biopsy sections were examined by three senior pathologists who were unaware of each other's observation. Whenever indicated, Pearl's stain was done in aspirated slides using 0.5% potassium ferrocyanide, 0.75% hydrochloric acid (HCL), and nuclear fast red as counterstain. Bone marrow iron is graded using Gale's grading system.^[8] Reticulin staining was done in a few indicated cases using Gomori's method.^[9] The data collected were presented as tables and charts using Microsoft Word (2010) and Microsoft Excel (2010).

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study

protocol and the subject information and consent form were reviewed and approved by our institutional ethics committee according to the document number 5324(A) dated June 17, 2020.

RESULTS

A total of 42 patients of pancytopenia were evaluated. The age range was 19–80 years. The most common age group was 18–28 years. Twenty-four patients were male and 18 were female with a male-to-female ratio of 1.33:1.

Symptoms associated with pancytopenia

It is observed that the most common presenting symptom associated with pancytopenia was generalized weakness (42 cases, 100%) followed by abdominal discomfort (16 cases, 38.01% of all), fever with vomiting (seven cases, 16.67%), fever with jaundice (three cases, 7.14%), bleeding manifestation (three cases, 7.14%), fever with rash (two cases, 4.76%), and dyspnea (one case, 2.38%).

Distribution of clinical signs associated with pancytopenia

It is found that the most common clinical sign associated with pancytopenia was pallor (all cases, 100%) followed by hepatomegaly (20 cases, 47.62%), splenomegaly (eight cases, 19.05%), edema and enlarged lymph node (four cases each, 9.52% each), and icterus (three cases, 7.14%).

Hemoglobin

The range of hemoglobin was 3.2–9.8 g/dL, with a mean of 6.06 g/dL and standard deviation (SD) of 1.82 g/dL.

Total leukocyte count

The range of TLC was 900–2945/cmm with a mean of 1824 and SD of 574.

Platelet count

The range of platelet count was 20,000–95,000/cmm. The mean and SD are 48,750/cmm and 27,570/cmm, respectively.

Peripheral blood smear finding

Anisocytosis (38 cases, 90.48%), macrocytosis (21 cases, 50%), and hypersegmented neutrophils (20 cases, 47.62%) [Figure 1] were the most common findings in the peripheral blood smear. A significant number of cases (21 cases, 50%) presented with normocytosis, and a few cases (four cases, 9.52%) presented with dimorphic picture on peripheral blood smear (PBS). No cases of immature cells in PBS were found.

Bone marrow aspirate and biopsy finding

It is observed that 35 cases (83.33%) showed hypercellular marrow followed by five cases (11.90%) showing hypocellular marrow on bone marrow aspirate study. Also 35 cases (83.90%) showed erythroid hyperplasia, 34 cases (80.95%) showed normal range and the maturation

of myeloid series, and 36 cases (85.71%) showed normal megakaryocytic maturation. Five cases (11.90%) showed hypoplasia of all the three lineages [Table 1].

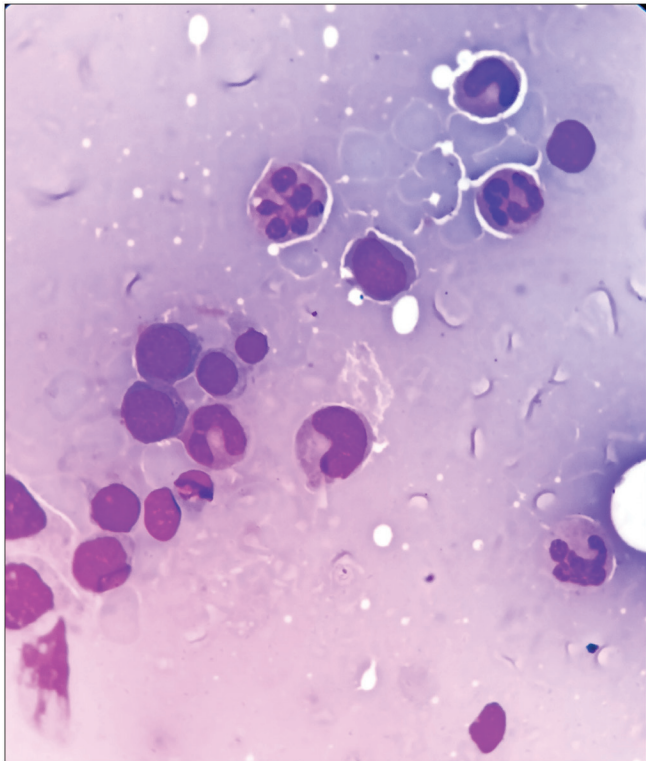


Figure 1: Bone marrow aspiration showing hypersegmented neutrophils in a case of megaloblastic anemia; oil (1000×), May Grunwald Giemsa stain

Other investigations

Twenty cases were evaluated for serum vitamin B12, and 15 cases (35.71%) showed a decreased serum vitamin B12 level. There were no cases with ring sideroblasts or increased bone marrow reticulin fibers.

Etiology of pancytopenia

It was found that megaloblastic anemia [Figure 2] was the most common cause of pancytopenia, which was followed by hypoplastic anemia [Figure 3], normoblastic erythroid maturation, and multiple myeloma [Figure 4]. No cases of subleukemic leukemia or aleukemic leukemia were found. One case of metastatic tumor with morphology of malignant small round cell tumor/lymphoma was found [Figure 5] [Table 2].

DISCUSSION

Clinical findings (signs and symptoms)

Symptoms

In the study conducted by Shah and Patel (2020), the most common clinical findings were generalized weakness and pallor found in all of the 145 cases (100%) followed by fever (55%), dyspnea (41%), hepatomegaly (14%), and splenomegaly (10%).^[10] In the studies conducted by Sharma *et al.* (2017), Deshpande *et al.* (2019), Gayathri and Rao (2011), and Suryareshmi *et al.* (2018), the most common symptoms associated with pancytopenia were generalized weakness complained by all of the 100 patients (100%), 74 of 101 patients (73.2%), all of the 100 patients

Table 1: Morphology on bone marrow aspiration in cases of pancytopenia			
Parameters	Findings	Number of cases (n)	Percentage (%)
Cellularity	Normocellular	2	4.76
	Hypocellular	5	11.90
	Hypercellular	35	83.33
Erythroid series	Normal	2	4.76
	Hypoplastic	5	11.90
	Hyperplastic	35	83.90
Myeloid series	Normal	34	80.95
	Hypoplastic	5	11.90
	Hyperplastic	3	7.14
Megakaryocytic series	Normal	36	85.71
	Hypoplastic	5	11.90
	Hyperplastic	1	2.38
M:E ratio	Normal/reversed	12/30	28.57/71.43
Myeloblast	Normal/increased	42/0	100/0
Promyelocyte	Normal/increased	42/0	100/0
Myelocyte	Normal/increased	42/0	100/0
Metamyelocyte	Normal/increased	42/0	100/0
Band cells	Normal/increased	12/30	28.57/71.43
Neutrophil	Normal/increased	34/3	80.95/7.14
Lymphocyte	Normal/increased	32/10	76.19/23.81
Eosinophil	Normal/increased	40/2	95.24/4.76
Plasma cell	Normal/increased	40/3	95.24/4.76

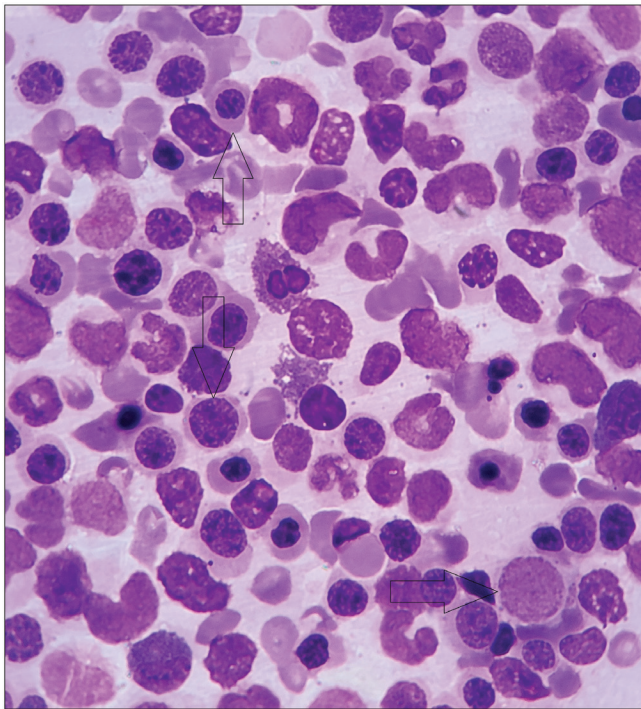


Figure 2: Bone marrow aspirate in megaloblastic anemia showing early megaloblasts (horizontal arrow), intermediate megaloblasts (downward arrow), and late megaloblasts (upward arrow); oil immersion (1000×), May Grunwald Giemsa stain

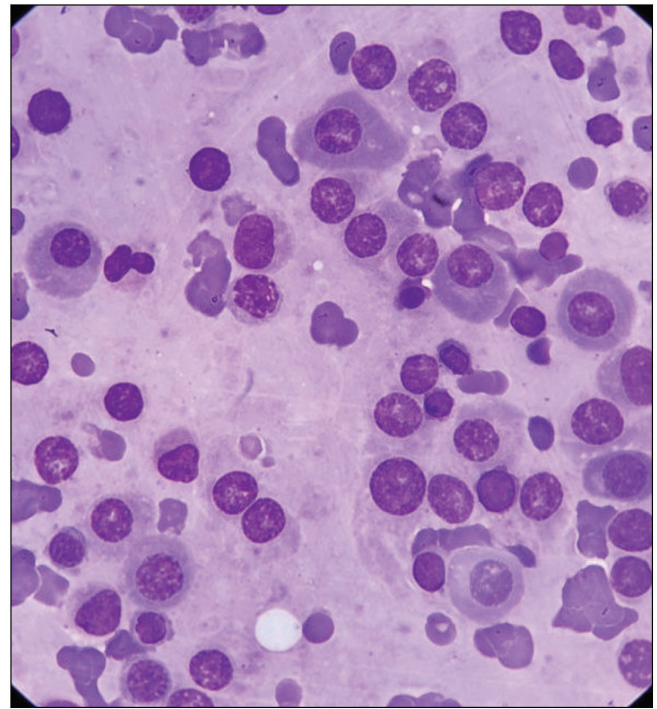


Figure 4: Bone marrow aspirate showing involvement by multiple myeloma with many atypical plasma cells; oil immersion (1000x), May Grunwald Giemsa stain

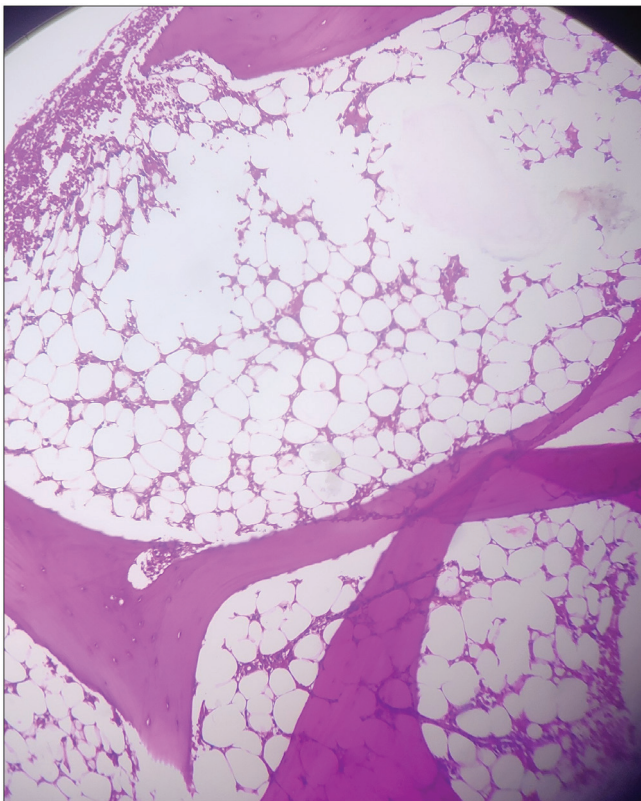


Figure 3: Bone marrow biopsy in aplastic anemia showing markedly hypocellular marrow; low power (100×), H&E stain

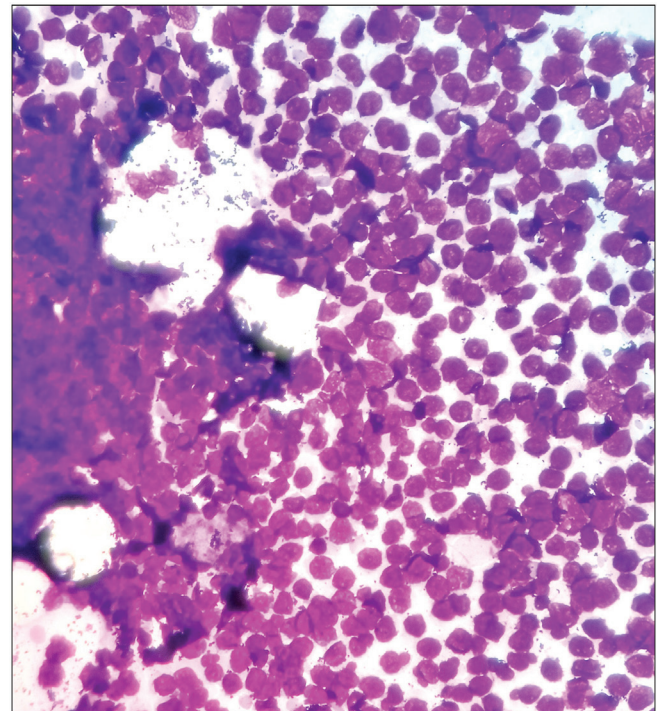


Figure 5: Bone marrow aspirate showing sheets of small round cells suggestive of metastasis of small round cell tumor/lymphoma; high power (400×), May Grunwald Giemsa stain

(100%), and 35 of 46 patients (76.10%), respectively.^[4,11-13] In the present study, most common clinical symptoms associated with pancytopenia were generalized weakness,

Table 2: Distribution of final diagnosis associated with pancytopenia with bone marrow study (aspiration and biopsy)

Etiology	Number of cases (n)	Percentage (%)
Megaloblastic anemia	26	61.90
Hypoplastic anemia	5	11.90
Multiple myeloma	3	7.14
Normoblastic erythroid hyperplasia	4	9.52
Suspicious of myelodysplastic syndrome	1	2.38
Metastasis (malignant small round cell tumor/lymphoma)	1	2.38
Normal study	2	4.76

which accounted for 100% with all the 42 patients affected by it, followed by abdominal discomfort (38.01%). This shows close resemblance to the study done by Shah and Patel (2020), Sharma *et al.* (2017), Deshpande *et al.* (2019), Gayathri and Rao (2011), and Suryareshmi *et al.* (2018).

Signs

In the studies conducted by Yadav and Kumar (2020), Shah and Patel (2020), Pereira and Dias (2016), and Sharma *et al.* (2017), the most common physical finding observed in the patients of pancytopenia was pallor with 94 out of total 94 cases (100%), 145 out of total 145 cases (100%), 80 out of total 80 cases (100%), and 100 out of 100 cases (100%), respectively, having it.^[10,11,14,15] In the present study, the most common clinical sign was pallor with all the 42 (100%) cases showing pallor as physical finding, and so the present study closely resembles to the study conducted by Yadav and Kumar (2020), Shah and Patel (2020), Pereira and Dias (2016), and Sharma *et al.* (2017).

Peripheral blood findings

In the studies conducted by Agarwal *et al.* (2018), Gayathri and Rao (2011), and Tilak *et al.* (1999), the most common PBS finding was anisocytosis found in 94%, 60%, and 83.1% cases, respectively.^[13,16,17] In the present study, anisocytosis was the most common PBS finding found in 90.48% of cases and thus showing resemblance to the studies mentioned above. Agarwal *et al.* (2018) and Sweta *et al.* (2014) found macrocytosis as one of the common PBS findings with a prevalence of 45% and 49%, respectively.^[16,18] The present study showed macrocytosis in 50% of cases, which is similar to the above studies.

Bone marrow study

The cellularity of bone marrow was evaluated on the basis of bone marrow aspiration study, and few of the cases were confirmed by a biopsy study with age taken in consideration. In studies conducted by Agarwal *et al.* (2018), Govindaraj *et al.* (2015), Varma *et al.* (2018), Gandhi *et al.* (2019), and Raina *et al.* (2020), hypercellularity due to erythroid hyperplasia with megaloblastic changes was the most common bone marrow findings (78.75%, 76%, 56.5%, 67.91%, 81.35%, respectively) followed by hypocellularity (10%, 10%, 10.3%, 14.9%, 15.25%, respectively).^[11,16,19-21] In the present study, the most common finding in bone

marrow study was hypercellularity with 35 cases out of total 42 cases (83.33%), followed by hypocellular marrow with five cases out of total 42 cases (11.90%), and thus showing resemblance with the studies performed by Agarwal *et al.* (2018), Govindaraj *et al.* (2015), Varma *et al.* (2018), Gandhi *et al.* (2019), and Raina *et al.* (2020).

Etiological diagnosis of pancytopenia

In a study conducted by Shwetha *et al.* (2021), the most common cause of pancytopenia was megaloblastic anemia (39%) followed by aplastic/hypoplastic anemia (12.17%).^[22] In other studies conducted by Mittal *et al.* (2019), Batool *et al.*, Sweta *et al.* (2014), Shah *et al.* (2020), and Gayathri and Rao (2011), the most common causes of pancytopenia, after proper hematological and biochemical evaluation, were megaloblastic anemia (with 33.34%, 27%, 66%, 32%, 77.04% of all cases, respectively) followed by aplastic anemia (with 19.05%, 15.6%, 18%, 16%, 18.26% of all cases, respectively).^[10,13,18,23,24] In a study conducted by Lakhey *et al.* (2012), the commonest cause of pancytopenia was hypoplastic anemia (29.6%), followed by hematological malignancies (27.78%).^[25] Mangal and Sinha (2020) found aplastic anemia (24.2%) as the most common cause followed by myelodysplastic syndrome (17.6%).^[26] Retief and Heyns (1976) concluded bone marrow failure (67.1%) as the commonest cause of pancytopenia followed by hypersplenism (17.7%).^[27]

In the present study, the most common cause of pancytopenia was megaloblastic anemia with 26 of total 42 cases (61.90%), followed by aplastic/hypoplastic anemia (11.90%), which is in concordance with studies by Shwetha *et al.* (2021), Mittal *et al.* (2019), Batool *et al.* (2021), Sweta *et al.* (2014), Shah *et al.* (2020), and Gayathri and Rao (2011), but in sharp contrast with the studies conducted by Lakhey *et al.* (2012), Mangal and Sinha (2020), and Retief and Heyns (1976) [Table 3]. Three cases of pancytopenia due to the marrow involvement by multiple myeloma were found in the present study. Bone marrow hypoplasia due to infiltration by multiple myeloma was also found by Medhi *et al.*^[28] One case of metastatic malignancy with features of malignant round cell tumor/lymphoma and one case suspicious of myelodysplastic syndrome were found in the present study. Although these are very significant diagnosis, frequency is not sufficient to compare with other studies or to make a comment.

Table 3: Comparison of common causes pancytopenia in different literatures

Studies	Number of cases	The first most common diagnosis	The second most common diagnosis
Shwetha <i>et al.</i> (2021), India	115	Megaloblastic anemia (67.82%)	Aplastic anemia (12.17%)
Mittal <i>et al.</i> (2019), India	42	Megaloblastic anemia (33.34%)	Aplastic anemia (19.05%)
Batool <i>et al.</i> (2021), India	237	Megaloblastic anemia (27%)	Aplastic anemia (15.6%)
Sweta <i>et al.</i> (2014), India	112	Megaloblastic anemia (66%)	Aplastic anemia (18%)
Shah <i>et al.</i> (2020), India	145	Megaloblastic anemia (32%)	Aplastic anemia (16%)
Lakhey <i>et al.</i> (2012), Nepal	54	Hypoplastic anemia (29.6%)	Hematological malignancies (27.785%)
Mangal and Sinha (2020), India	91	Aplastic anemia (24.2%)	Myelodysplastic syndrome (17.6%)
Retief and Heyns (1976), South Africa	195	Bone marrow failure (67.1%)	Hypersplenism (7.7%)
Gayathri and Rao (2011), India	104	Megaloblastic anemia (74.04%)	Aplastic anemia (18.26%)
Present study (2020–21), India	42	Megaloblastic anemia (61.90%)	Aplastic anemia/hypoplastic anemia (11.90%)

The comparison of common causes of pancytopenia in different literatures and the present study is presented in Table 3.

Vitamin B12 assay

In a study by Agarwal *et al.* (2018), 19 out of 30 cases of megaloblastic anemia were investigated for vitamin B12 assay, and all the cases (100%) showed serum vitamin B12 less than 211 pg/mL (211–946 pg/dL is the normal range in the study).^[16] In the present study, 20 of 26 megaloblastic anemia cases were evaluated for serum vitamin B12 level, 15 of which show low level (normal serum vitamin B12 range: 180–640 ng/L). Thus, it can be concluded that the most common cause of megaloblastic anemia in this part of country is nutritional deficiencies, mainly vitamin B12 deficiency, which closely resembles to the study done by Agarwal *et al.*^[16]

CONCLUSION

Generalized weakness and pallor were the most common clinical findings associated with pancytopenia followed by abdominal discomfort and hepatomegaly. Megaloblastic anemia was the most common etiology followed by hypoplastic/aplastic anemia. Although bone marrow aspiration is not essential to diagnose megaloblastic anemia, it is necessary to exclude other causes of pancytopenia such as hypoplastic/aplastic anemia, leukemia, plasma cell dyscrasia, metastasis, myelodysplastic syndrome etc. Bone marrow biopsy is essential in hypocellular yield in aspiration to confirm hypoplastic/aplastic anemia and also to confirm space occupying lesions such as metastasis. Plasma cell dyscrasia can present as pancytopenia, which always must be remembered when evaluating a case of unexplained pancytopenia.

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Conflicts of interest

There are no conflicts of interest.

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