Original article

Pancytopenia – Diagnosis on Bone Marrow Aspiration

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Abstract

Introduction – pancytopenia is a descriptive term referring to a reduction in all three blood cell lineage: erythrocytes, leukocytes & platelets. Pancytopenia can result from diverse mechanisms, as pancytopenia can be associated with a decrease in hematopoietic cell production in the bone marrow secondary to toxins, medications, or infection. In such cases, the bone marrow appears hypoplastic. In other situations, the marrow may appear normocellular or even hypercellular.

Aims: To study the clinical presentations in pancytopenia due to various causes; and to evaluate hematological parameters, including bone marrow aspiration.

Materials and Methods: It was a prospective study, and 52 pancytopenic patients were evaluated, along with hematological parameters and bone marrow aspiration during the period of 2 years, from July 2013 to Sept 2015, at Hematology Unit, Department of Pathology.

Results: Among 52 cases studied, age of patients ranged from 03 to 70 years, and male predominance. The commonest physical finding was pallor, followed by fever and weakness. Bone marrow aspiration was conclusive in all cases. The commonest marrow finding was hypocellularity with aplastic anemia. The commonest cause for pancytopenia was aplastic anemia(38.5%) followed by myelodysplastic syndrome(11.5%) & megaloblastic anemia(9.6%).

Conclusion: The detailed primary hematological investigations along with bone marrow aspiration in pancytopenic patients are helpful for understanding disease process and to diagnose or to rule out the causes of pancytopenia. These are also helpful in planning further investigations and management.

Keywords: Bone marrow aspiration, pancytopenia, aplastic anemia. Myelodysplastic syndrome

Introduction

Pancytopenia is a disorder in which all three major formed elements of blood (red blood cells, white blood cells and platelets) are decreased in number **(1).** It is not a disease entity but a triad of findings that may result from a number of disease processes — primarily or secondarily involving the bone marrow **(2).** The severity of pancytopenia and the underlying pathology determine the management and prognosis of the patients **(3).** In India, the causes of pancytopenia are not well defined, so the present study has been undertaken to evaluate the various causes and to correlate the peripheral blood findings with bone marrow aspirate **(3,4).** Thereby, this data would help in planning the diagnostic and therapeutic approach in patients with pancytopenia.

Bone marrow – Diagnosis & management of many hematologic diseases depend on examination of the bone marrow. The cytology preparation of bone marrow cells obtained by aspiration of the marrow & a smear of the cells, allowing excellent visualization of the morphology & enumeration of the cells. **(5)**

Objectives: To study the clinical presentations in pancytopenia due to various causes; and to evaluate

haematological parameters, including bone marrow aspiration.

Materials and methods

The present prospective study was undertaken for a period of 2 years, from July 2013 to Sept 2015, at Hematology Unit, Department of Pathology. Patients of all age groups and both sexes were included. Case selection was based on clinical features and supported by laboratory evidence, which included peripheral blood counts for hemoglobin, leukocytes and platelets. Inclusion criteria were presence of all 3 of the following: hemoglobin, <9 g/dL; total leukocyte count (TLC), <4,000 / µL ; platelet count, <100,000/ µL (6).

Bone marrow aspiration (BMA) is a reliable and rapid method of marrow evaluation. There are several indications for examination of bone marrow. These include further workup of hematologic abnormalities observed in the peripheral blood smear, evaluation of primary bone marrow tumors, staging of bone marrow involvement by metastatic tumors, assessment of infectious diseases processes including fever of unknown origin & evaluation of metabolic storage diseases.

Bone marrow aspirate smear allows cytological examination of bone marrow cells.

Patients on myelotoxic chemotherapy were excluded. Two milliliters of EDTA (ethylene diamine tetra-acetic acid) anticoagulated blood was collected and processed through automated hematology analyzer; and nine hematological parameters were obtained, which included hemoglobin, red blood cell count, total leukocyte count, differential leukocyte count, platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), packed cell volume (PCV). Peripheral smear was stained by field stain for all the cases and examined in detail. Bone marrow aspiration was subsequently carried out under aseptic precaution after obtaining written consent from the patient or guardian.

Subject population

From June 2013 to July 2015, 52 patients were recruited in the study and underwent bone marrow aspiration.

BMA was done usingJamshidi needle and aspirate was withdrawn with a 10 ml plastic syringe from posterior superior iliac spine. Aspirated material was delivered onto clean glass slides and smears were prepared immediately. Peripheral smears and marrow aspirate smears were stained by Leishman & field stain.

Inclusion Criteria for this Study Were

1. Hemoglobin level - below 13.5 g/L for males andbelow 11.5 g/L for females
2. Total leucocyte count - below 4 × 109/L
3. Platelet count - below 150 × 109/L.

Results

A total of 52 patients who presented with pancytopenia were studied. They consisted of 28 males and 24 females with a male-to-female ratio of “1.15:1”. Eleven patients were of pediatric age group and rest 41 are adults. The age of patients ranged from 03 to 70 years. No familial disease was observed in association with pancytopenia.

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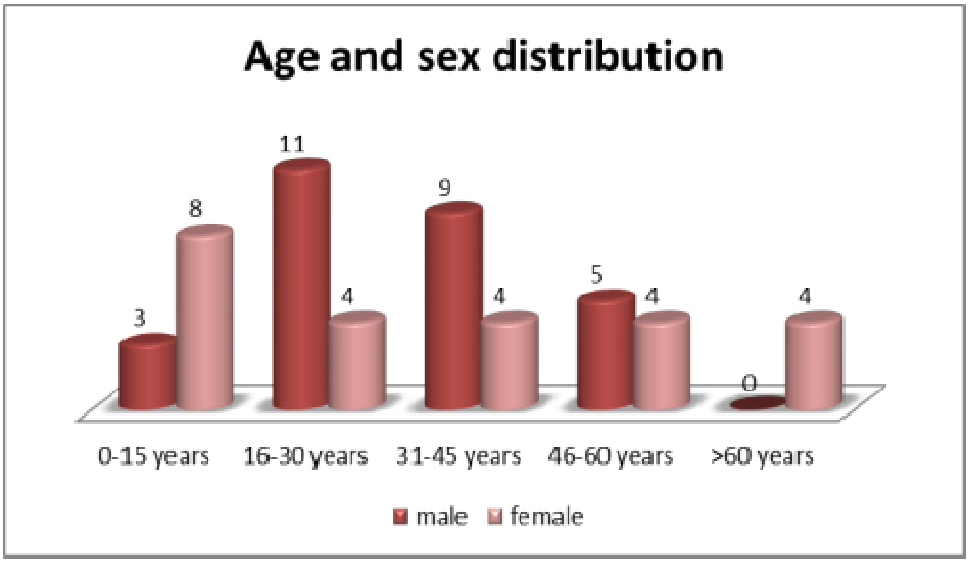
Graph 1:



Most of the patients (15) are of age group 16 to 30 years followed by 13 patients of age between 31 to 45 years. Four patients were more than 60 years of age.

Table 1:

presenting complaints & physical finding in pancytopenis	Number of cases	Percentage (%)
generalized weakness	27	45.65
Dyspnea	2	4
Pallor	52	100
Fever	31	56
Bleeding manifestation	6	13
abdominal pain	9	15
weight loss	1	2
Rashes	3	6
Bodyache	10	21
ear discharge	1	2
Jaundice	3	6
Splenomegaly	13	28
Hepatomegaly	5	10
Lymphadenopathy	7	13.5

Table 1 : Presenting complaints and physical findings in pancytopenia

Presenting complaints and physical findings are shown in Table 1. Pallor was most common finding followed by fever, generalized weakness, splenomegaly, bodyache, abdominal pain, bleeding manifestation and other. The commonest mode of presentation was generalized weakness; other main symptoms were dyspnea, fever, weight loss. Pallor was noted in all cases.

Splenomegaly and hepatomegaly were seen in cases of megaloblastic anaemia, followed by sub leukemic leukaemia and malaria. Lymphadenopathy was noted in sub leukemic leukaemia – lymphoblast type.

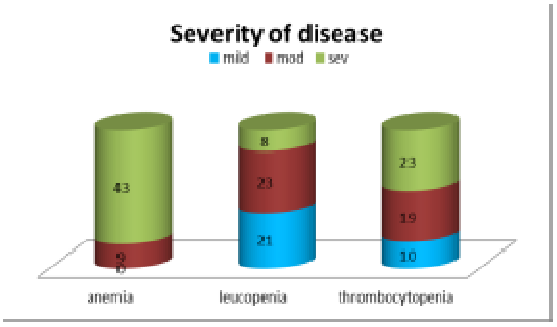
Table 2:

Cause	Number of cases	Percentage
Megaloblastic anemia	5	9.60%
PRCA	1	1.90%
Aplastic anemia	20	38.50%
acute lymphoblastic leukemia	5	9.60%
Myelodysplastic syndrome	6	11.50%
acute myeloid leukemia	2	3.80%
hemophagocytic syndrome	4	7.70%
Non Hodgkin lymphoma	5	9.60%
storage disorder	1	1.90%
Hypersplenism	2	3.80%
Malaria	1	1.90%
Total	52	100%

Table 2: The causes of pancytopenia and case distribution

Most common cause of pancytopenia in our study was aplastic anaemia (38.5%) followed by myelodysplastic syndrome(11.5%), megaloblastic anaemia (9.6%), non-Hodgkin's lymphoma (9.6%), hemophagocytic syndrome (7.7%) and other shown in table 2.

Graph 2



(mod- moderate, sev- severe)

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Peripheral smear was studied and all lineage were described in mild, moderate and severe categories. 43 out of 52 patients have severe anaemia followed by nine moderate anaemia. Eight patients have severe leukopenia, 23 have moderate and 21 have mild leukopenia. Twenty three patients have severe thrombocytopenia, nineteen have moderate and ten have mild thrombocytopenia.

Table:3

Cellularity	Number of cases	Percentage
hypercellular	20	38%
Normocellular	7	14%
Hypocellular	25	48%
Total	52	100%

Table 3 case distribution according to cellularity

Bone marrow examination revealed hypocellular marrow is most common finding (48%), followed by hypercellular marrow in 38% cases and normocellular marrow in 14 % cases.(Table 3)

Aplastic anaemia was seen in twelve males and eight females; their age ranged from 11 to 70 years, with a mean age of 40 years. In the present study, out of 20 cases of bone marrow hypoplasia, cause was not known in 18 cases and was grouped under idiopathic bone marrow hypoplasia. One patient had history of presinusoidal cirrhosis. Another patient gave history of treatment with antibiotics for urinary tract infection. Bone marrow (BM) showed hypocellularity with suppression of erythropoiesis, myelopoiesis and megakaryopoiesis with relative lymphocytosis [Figure 1].

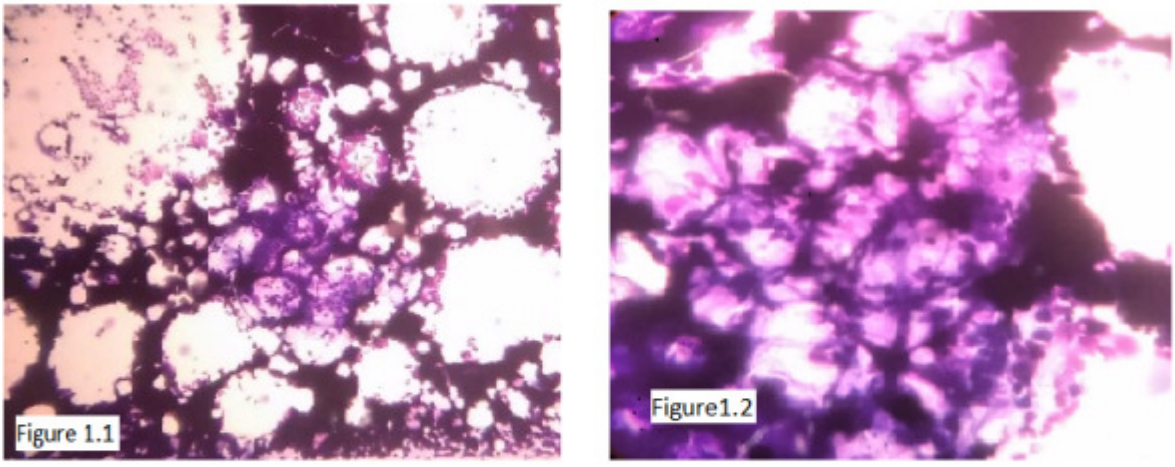

Figure 1.1 (10X) & figure 1.2 (40X) : Aplastic anemia- Bone marrow showing hypocellularity with increased fat and reactive lymphocytosis

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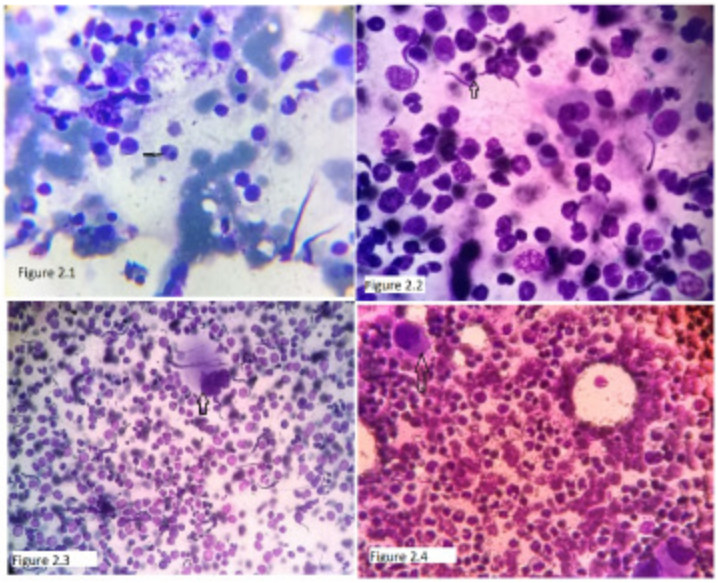
In the present study, Myelodysplastic syndrome (MDS) was observed in six patients out of this two males and four females, their age ranging from 13 to 54 years, with a mean age of 33 years. The cause was not known in all cases. Similarly, hypocellular & hypercellular MDS, can present with pancytopenia, and many of these syndromes are defined, at least in part, by their cytopenia. Primarily a disease of adults, MDS is commonly characterized by progressive bone marrow failure, with several of the subtypes often progressing to acute myeloid leukaemia (AML). Not unexpectedly, the more "high-grade" MDS categories that demonstrate extensive bone marrow failure, such as refractory cytopenia with multilineage dysplasia and refractory anaemia with excess blasts, more commonly present with pancytopenia. In present study we detect three cases of hypercellular MDS and three cases of hypocellular MDS. The aspirate smears typically demonstrate varying levels of dysplasia in one or more cell lines. An interesting diagnostic dilemma in the setting of new-onset pancytopenia can be differentiating hypoplastic MDS from AA, both can present with profound hypocellularity, but few features can help in the differentiation. MDS often presents with dyserythropoietic RBCs, dysplastic granulocytes, and hypogranular platelets, which are not often seen in the setting of AA.

[Figure 2]



Megaloblastic anemia was observed in three males and two females, their age ranging from 15 to 47 years, with a mean age of 31 years. In the present study, out of five cases of bone marrow hyperplasia, cause was not known in all cases. B12 levels were estimated in two cases & found low. In all cases both folic acid and parenteral hydroxycobalamine therapies were administered, and they showed complete clinical and hematological remission. Bone marrow aspiration showed megaloblastic erythroid hyperplasia.

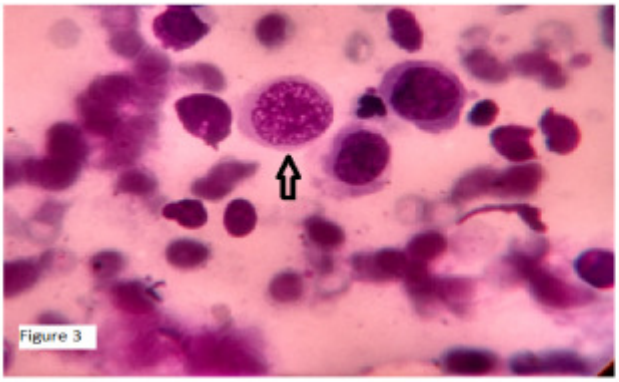


Figure 3: Bone marrow showing megaloblasts, with royal blue cytoplasm and sieve-like chromatin

We encountered eight patients of sub leukemic leukaemia; their age ranged from 03 to 54 years. Two cases were of AML-M2 (acute myeloblastic leukaemia) , one case was AML-M1 and three cases were of ALL (acute lymphoblastic leukaemia). Bone marrow was hypercellular in all cases. Erythroid and megakaryocytic series were reduced. Majority of cells were myeloblasts and lymphoblasts, constituting more than 50% and 40% of cells in marrow, respectively. Bone marrow aspirate showed myeloblastic proliferation [Figure 4] in acute myeloid leukaemia. Bone marrow show lymphoblastic proliferation [Figure 5] in case of acute lymphoblastic leukaemia.

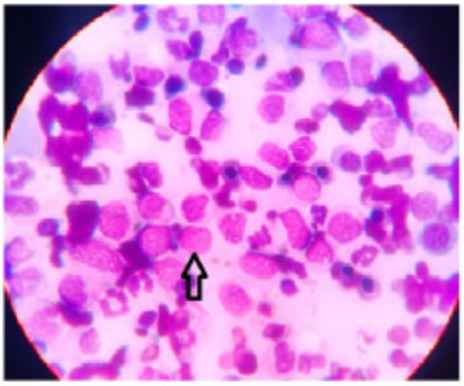


Figure 4: Bone marrow showing myeloblasts case of acute myeloid luekaemia

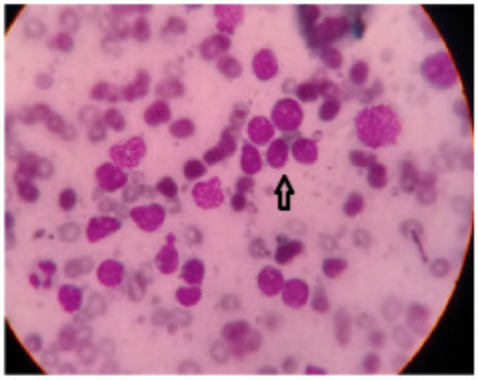


Figure 5 : Bone marrow showing lymphoblasts, a case of Acute Lymphoblastic Leukemia (ALL)

Malarial infestation was seen in one female patient aged eight years . Peripheral blood picture showed pancytopenia, and gametocytes of plasmodium falciparum were seen in blood smear. Bone marrow was normocellular with hemophagocytic changes. No malarial parasites were seen on bone marrow smears. The patients recovered after antimalarial treatment and folic acid therapy.

Storage disorder was diagnosed in a 20 years old male, who presented with fever, pallor and hepatosplenomegaly. Bone marrow showed good number of large cells with peripherally placed relatively small nucleus and abundant multivacuolated foamy cytoplasm . Hence diagnosis of Gaucher's disease was considered.

Discussion

Pancytopenia is not an uncommon haematological problem encountered in clinical practice and should be suspected on clinical grounds when a patient presents with unexplained pallor, prolonged fever, and tendency to bleed. Bone marrow examination is a frequently requested investigation to determine the cause pancytopenia.

A total of 52 patients presenting with pancytopenia were included in the study. The age of the patients ranged from 03 to 70 years with a mean age of 36 years and males (53.84%) and females (46.15%), a male: female ratio of "1.16:1". Age and sex distribution of the patients were compared to similar data in the literature (Table 4). A total of 52 cases of pancytopenia were studied. Age, gender-wise incidence, presenting complaints, peripheral blood picture, bone marrow aspiration smears and various causes of pancytopenia were studied in all cases, and observations were compared with those in studies published in the literature.

Table 4

S.no	Authors	No. of cases	Age range	M:F
1	Khunger JM et al[7] (2002)	200	2-70	1.2:1
2	Tilak V et al.[3] (1992)	77	5-70	1.14:1
3	Kumar R et al.[6] (2001)	50	3-69	1.3:1
4	Present study	52	3-70	1.16:1

Age, sex distribution compared to those in other studies of pancytopenia

Pallor is a common clinical presentation in patients with pancytopenia and is present in all patients. In our study, fever and generalized weakness were the other common clinical findings which were seen in 56% (31/52) and 45.62% (27/52) cases, respectively. Vandana et al find generalized weakness (51%) as the most common symptom followed by fever (27%) and bleeding manifestations (11.7%). In a study by Khodke et al . fever (40%) was the commonest symptom, followed by weakness (30%) and bleeding manifestation (20%). In another study by Niazi and Raziq weakness (68.2%) was the commonest symptom, followed by fever (47.7%) and bleeding manifestations (33.7%)(8).The commonest clinical sign that we came across in both adults and children was pallor (100%), followed by splenomegaly (28%). In studies conducted by Khodke et al. and Niazi and Raziq, pallor and hepatosplenomegaly were the commonest sign, as in the present study. Similar

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features were noted in studies done by Tilak and Jain, Khunger et al. and Nanda et al**.(4,7,9)**

The commonest cause of pancytopenia, reported in various studies has been megaloblastic anaemia. This is in sharp contrast with the results of our study, where the commonest cause of pancytopenia was found to be aplastic anaemia (38%) followed by myelodysplastic syndrome(11.5%) and megaloblastic anaemia(9.6%) .Study done by Tilak and Jain, Khodke et al., Khunger et al., Gayathri and Rao, and Manzoor et al. who in their studies found megaloblastic anaemia, 68%, 44%, 72%, 74%, and 56% respectively as the most common cause of pancytopenia, followed by aplastic anemia 7.7%, 14%, 14%, 18% and 14% respectively. Kumar et al. ,Naseem et al. in India and Niazi and Raziq et al. have enumerated the most common cause of pancytopenia as aplastic anaemia, followed by megaloblastic anaemia respectively**(4,8).** Incidence of aplastic anaemia varies from 10% to 52% among pancytopenic patients**(7).** The incidence of hypoplastic anaemia in our study was 38.5%, which correlated with the corresponding figures in studies done by Kumar R et al. 29.5%, A lower incidence, viz., was reported by Khodke K et al. and Khunger JM et al. Both observed an incidence of 14%. **(6,7)**

In our study, acute lymphocytic leukaemia accounted for 9.60% of the cases of pancytopenia, Acute myeloid leukaemia constituted 3.8% cases of pancytopenia which is low compared to a similar study by Jha et al. (19.59%) in contrast to a study by Kumar R et al. reported five cases of ALL, 13 cases of AML, two cases of hairy cell leukaemia out of 166 cases of pancytopenia, over a six years study period **(10, 4).** In other studies by Hirachand et al. and Pathak et al. acute leukaemia accounted for 7.69% and 8.8%, respectively. Khodke K et al. reported a single case of AML-M2 out of 50 cases of pancytopenia**(7).**

Non-Hodgkin lymphoma was other haematological malignancy seen in adult. Similar to other studies, this malignancy was not seen in a significant number. Haemophagocytic syndrome was seen in 7.70% (4/52) cases of pancytopenia.

PRCA (Pure red cell erythroid aplasia) was seen in 1.9% (01/52) case of pancytopenia in this study. One case of a storage disorder 1.90% (1/52) was also detected in bone marrow aspiration. Normal bone marrow in pancytopenia was seen in 3.80% (2/52) cases. Similar data has been obtained by Jha et al.11 (3.38%) and Pathak et al.17 (5.8%). **(10)**

We encountered one case of malaria in our study, constituting 1.9% of total cases – compared to Khunger JM et al., who have reported an incidence of 1%; Tilak V et al., who have reported an incidence of 3.9%; and Kumar R et al., who have reported an incidence of 03% of the total cases**(3,4,6).**

We have reported a single case of storage disorder (Goucher's Disease), in a 15-year-old boy, who presented with hepatomegaly, splenomegaly and pancytopenia. BM was normocellular with normoblastic erythropoiesis. Aspirated smears showed collection of Goucher's like cells dispersed throughout the smear. Kumar R et al., Khunger JM et al. and Khodke K et al. have not reported any case of storage disorder as a cause of pancytopenia in their studies**(4,6,7).**

Normal bone marrow can be seen in pancytopenic patients as a result of sequestration and/or destruction of cells by the trapping of normal cells in a hypertrophied and over-reactive reticuloendothelial system known as hypersplenism.

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Conclusion

Pancytopenia is a common hematological problem encountered in clinical practice and common cause of pancytopenia in our study is aplastic anemia . Aplastic anemia is a medical emergency & warrants an early workup and prompt treatment. The present study concludes that detailed primary hematological investigations along with bone marrow aspiration in cytopenic patients are helpful for understanding the disease process; to rule out the causes of, cytopenia; and in planning further investigations and management of cytopenia patients.

Footnotes

Source of Support: Nil

Conflict of Interest: None declared.

Acknowledgement -I would first like to thank Prof. Dr. Patel & Dr. Prof. Sharad Thora sir , he helped me in starting bone marrow aspiration in pathology department. The door to Dr. Manoj Gupta sir was always open whenever I ran into a trouble spot or had a question about my reporting.

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