



Original Research Article

Evaluation of bone marrow in patients with pancytopenia

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ABSTRACT

Introduction : Pancytopenia is common hematological term described as decreased red blood cells, white cells and platelets in blood caused by either reduced production of haematopoietic cells in bone marrow or secondary destruction or reduction by drugs, toxins, radiotherapy, chemotherapy, infection etc. The diagnosis of causes for it are difficult to decide but few minimal invasive methods like bone marrow aspiration(BMA) and biopsy (BMB)are very useful.

Objectives: To evaluate the diagnostic role of BMA and BMB in pancytopenia.

Materials and Methods: This was observational study conducted over a period of 36 months on 145 patients. All age patients with new onset pancytopenia fulfilling inclusion criteria were included. Exclusion criteria: Radiation/chemotherapy induced pancytopenia. BMA and BMB were done in all cases. BMA smears were air dried and stained by Romanowsky stains while biopsy was fixed in formalin and sent for paraffin sectioning. BMA and BMB were analysed and data was statistically analysed.

Results: Out of 145 patients, The mean age of the patients was 46years.Pallor and generalized weakness were commonest clinical complaints. Megaloblastic anemia was the most common cause (32%) followed by hypoplastic marrow in 16% cases. Rest causes were acute leukemia, anemia, myelodysplastic syndrome, metastases etc.

Conclusion: BMA is good for studying morphology of hematopoietic cells while BMB for assessing cellularity and structure of bone marrow like fibrosis, infiltration & metastasis. BMA and BMB are complimentary with each other when used together for evaluation of bone marrow in routine haematological disorders.

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1. Introduction

Pancytopenia is common hematological term described as decreased red blood cells, white blood cells and platelets caused by either reduced production of haematopoietic cells in bone marrow or secondary destruction or reduction by drugs, toxins, radiotherapy, chemotherapy, infection etc.^{1,2}Laboratory parameters are-haemoglobin< 9 gm/dl, WBC < 4,000/cmm, and platelets < 1,00,000/cmm.¹ Clinical signs and symptoms are pallor, fatigue, dyspnoea, bleeding or bruising, susceptibility to infection, organ and lymphnode enlargement.¹

The diagnosis of pancytopenia is challenge and requires cooperation of both clinician and pathologist.³ It also give comments on disease stage, progression if any, management & prognosis.⁴The clinician has to apply comprehensive approach by detailed analysis of history, laboratory parameters and bone marrow study to reach correct cause of new onset pancytopenia.

BMA and BMB are complimentary with each other when used together for evaluation of bone marrow in routine haematological disorders. This study is done to statistically analyze the diagnostic value of both procedures and the lacuna observed when any one of the procedure performed alone.

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2. Materials and Methods

2.1. Study design

This was prospective and retrospective study of 145 cases of pancytopenia conducted at pathology department of a tertiary care hospital, Ahmedabad, Gujarat, India, over a period of 36 months (January 2016-December 2018). The study was held after approval from Institutional Ethical Committee (GCSMC/EC/TRIAL/APPROVE/2017/18).

2.2. Study groups

All the patients with different age at presentation that diagnosed as new onset pancytopenia in various clinical departments of hospital during this time period were identified through clinical case records to assess whether patient met the study inclusion criteria.

Inclusion Criteria were –hemoglobin < 10 gm%, total leukocyte count < 4000/cumm, platelet count < 1,50,000/cumm, new onset pancytopenia, all age groups and both sex.

Exclusion Criteria were-history of chemotherapy &/or radiotherapy and known cases of pancytopenia.

2.3. Study methods

Informed written consent was taken for BMA and BMB from the patient or guardian if minor. Peripheral smear, reticulocyte count, blood indices, prothrombin time, HIV, HBsAg tests were done before procedure. BMA was done using Salah needle no.16 for adults and no.18 for children with aseptic precautions. Aspirate was withdrawn with a 10 ml disposable plastic syringe from posterior superior iliac spine. 8-10 aspirate smears were prepared, air dried (fixed whenever required) and later stained by Leishman, Giemsa or Field stain. BMB was also done in all cases at same site by using Jamshidi needle with trocar and cannula. Biopsy material (atleast 1.5-2cm long) was sent to histopathology lab in 10% neutral buffered formalin, decalcified in 5% nitric acid, processed in routine paraffin embedding, 4-5µm thick sections were cut by Leica RM 2125 rotatory manual microtome. The slides were stained by Hematoxylin and Eosin (H&E) and then reported by pathologist using Nikon E 200 Light Microscope.

2.4. Data collected

All data including age, sex, hemoglobin, reticulocyte count, total white cell and platelet counts, clinical signs and symptoms, BMA, BMB diagnosis were collected from clinical case records of patients.

2.5. Statistical analysis

Data was entered in Microsoft excel sheets in computer. The statistical calculations were performed using a Statistical

Package for the Social Sciences Version 21 (SPSS, IBM company, Chicago, USA). The results were analysed using frequency distribution. The cases were also distributed according their age, sex and presenting clinical signs and symptoms. BMA and BMB findings were also compared and analyzed.

3. Results

Out of 145 patients, 137 were adults and 8 were children. The mean age of the patients was 46 years. 35 cases fall in to age group of 41-50 years. (Figure 1) The male to female ratio (1.1:1) was almost equal. (Figure 2) Pallor and generalized weakness were universal clinical complaints noted in all cases. The rest were fever (55%) and dyspnoea (41%). 14% and 10% patients were having enlargement of spleen and liver. (Figure 3)

The distribution of various causes of pancytopenia was shown in Table 1. The incidence of megaloblastic anemia was - 32% most frequent followed by hypoplastic marrow in 16% cases. Hypercellular bone marrow was noted in megaloblastic anemia, acute leukemia, Myelodysplastic syndrome (MDS), reactive conditions, lymphoma, immune thrombocytopenic purpura (ITP) and few cases of myeloproliferative neoplasm (MPN). However bone marrow cellularity was decreased in cases of pancytopenia caused by aplastic anemia, metastasis of unknown primary and few cases of myelofibrosis. Myeloid / Erythroid ratio (M/E ratio) was reversed in all cases of megaloblastic anemia and reactive conditions; but M/E ratio was increased in cases of Acute leukemias; however it was normal in cases of hypoplastic and aplastic anemia.

Megaloblastic anemia was observed in 46 adults with their age ranging from 10 years to 72 years. Out of them, 60% had decreased levels of vitamin B12 and folic acid. Peripheral smear showed moderate anisopoikilocytosis with predominantly macrocytic normochromic red cells, few macro-ovalocytes and hypersegmented neutrophils. Bone marrow aspiration showed hypercellular marrow with megaloblastic erythropoiesis. Megaloblasts had dyserythropoiesis, sieve like nuclear chromatin and arrested nuclear-cytoplasmic maturation. (Figure 4)

BMA smears of hypoplasia (17%) were diluted with peripheral blood and showed moderate reduction of hematopoietic precursors with increased marrow fat. There were focal cellular areas of lymphocytes and plasma cells. There were 4 cases of dry tap on BMA, later reported as acute leukemia (2), aplastic anemia (1) and myelofibrosis (1) on BMB. BMA cellularity was inadequate for reporting in 4 cases of pancytopenia, later reported as aplastic anemia (1), acute leukemia (1), nonhodgkins lymphoma (NHL) (1) and myelofibrosis (1).

BMA smears of acute leukemia were hypercellular with severe reduction of erythroid, myeloid and megakaryocytic series with presence of >20% blasts. (Figure 5) Six cases

of plasma cell myeloma showed increased number of lymphoid cells and presence of atypical plasma cells with hypo or hypercellular marrow.(Figure 6) Patients with bone marrow involvement by lymphoma showed severe reduction of all three lineages with presence of medium sized lymphoid cells. A possibility of bone marrow involvement by lymphoproliferative disorder was suggested in those cases and later proven as low grade nonhodgkins lymphoma by Immunohistochemistry on BMB.(Figure 7) One case of BMB showed presence of focal granulomas indicating tuberculosis as cause of pancytopenia. This was wrongly reported as megaloblastic anemia on BMA. In infective causes, one case of plasmodium falciparum malaria was detected with presence of gametocytes in the aspiration smears.

Table 1: Causes of cytopenia on bone marrow aspiration (BMA)

Diagnosis	No. of cases	% of cases
Megaloblastic anemia	49	34%
Hypoplastic anemia	33	24%
Normocellular/ Reactive marrow	14	10%
Plasma cell myeloma	6	4%
Anemia	12	9%
Acute leukemia	9	7%
Hypersplenism	2	1%
Myelodysplastic syndrome (MDS)	3	2%
Myelofibrosis	2	1%
Idiopathic thrombocytopenic purpura	2	1%
Metastases	2	1%
Malaria	1	1%
Lymphoma	2	1%
Dry tap	4	2%
Inadequate	4	2%
Total cases	145	

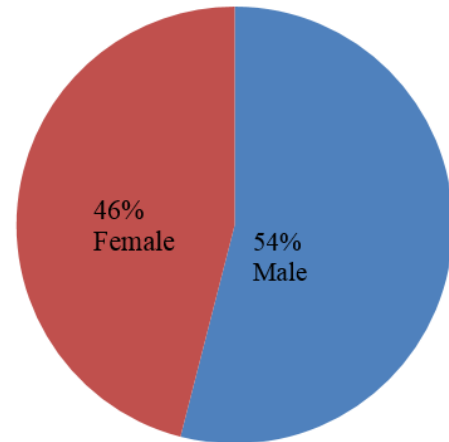


Fig. 2: Sex wise distribution of cases

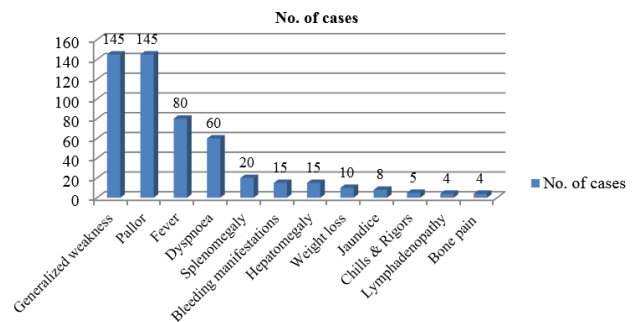


Fig. 3: Complaint wise distribution of cases

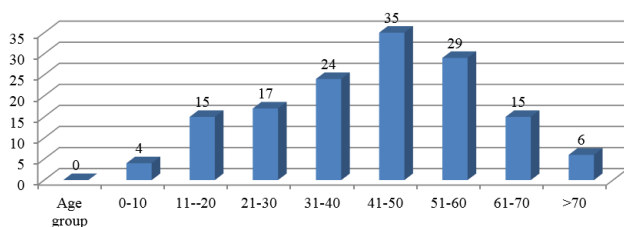


Fig. 1: Age wise distribution of cases

4. Discussion

The clinician finds pancytopenia very frequently in his daily out patient departments. It results due to reduced production of red cells, white cells and platelets in bone marrow or secondary to infection, drugs-chemotherapy, radiotherapy, cancer, fibrosis etc. ¹

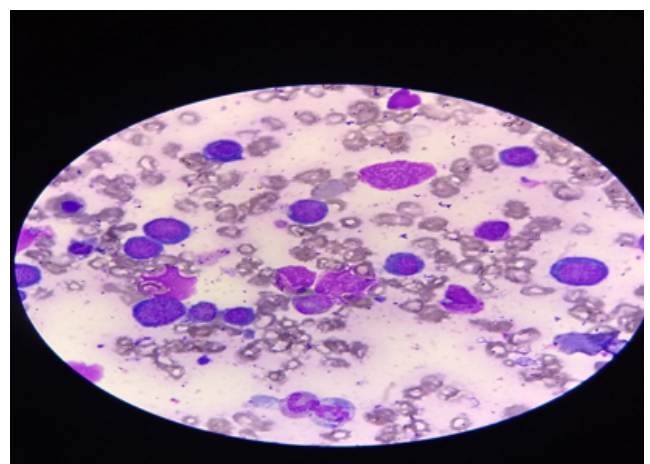


Fig. 4: Bone marrow aspiration smear of megaloblastic anemia (100x, Field stain)

Table 2: Comparison of findings between different studies

S.No	Study	Country	year	No. of cases	Commonest cause	Second most common cause
1	Das R et al	India	2016	64	Megaloblastic Anemia (58%)	Aplastic Anaemia (19%)
2	Singh et al	India	2017	150	Megaloblastic Anemia (49.3%)	Aplastic Anaemia (16%)
3	Jha et al	Nepal	2010	102	Hypoplastic Anaemia (42.15%)	Haematological Malignancy (19.4%)
4	Gayathri et al	India	2011	104	Megaloblastic Anemia (74.0%)	Aplastic Anaemia (18.3%)
5	Pereira A et al	India	2016	80	Megaloblastic Anemia (60%)	Aplastic Anaemia(10%)
6	Bahal N et al	India	2016	129	Megaloblastic Anemia (37%)	Haematological Malignancy (21%)
7	Rangaswamy M et al	India	2012	100	Megaloblastic Anemia (33%)	Nutritional Anaemia (16%)
8	Sweta et al	India	2014	100	Megaloblastic Anemia (66%)	Aplastic Anaemia (18%)
9	Kumar V et al	India	2018	210	Megaloblastic Anemia (62%)	Aplastic Anaemia (25%)
10	Present study	India	2019	145	Megaloblastic Anemia (32%)	Hypoplastic Anaemia (16%)

Table 3: Comparison of findings between BMA and BMB

Diagnosis	No. of cases BMB	Finding same as BMA	percentage %
Megaloblastic anemia	46	46	32
Hypoplastic anemia	25	25	17
Anemia	12	12	9
Normocellular/ Reactive marrow	17	14	12
Plasma cell myeloma	6	6	4
Acute leukemia	12	9	9
Myelofibrosis	4	2	3
Hypersplenism	2	2	1
Myelodysplastic syndrome (MDS)	3	3	2
ITP	2	2	1
Lymphoma	3	2	2
Metastases	5	2	3
Tuberculosis	1	0	0.5
Malaria	1	1	0.5
Aplastic anemia	6	0	4
Total Cases	145	126	

A total of 145 cases of pancytopenia were studied. The cases were studied for age and gender distribution, clinical complaints, BMA and BMB results. The findings were compared with other studies. The age of the patients ranged from 4 to 75 years, with a mean age of 46 years.(Figure 1) This findings were compared with other studies.⁴⁻⁶ Pancytopenias was observed more in males (54%) than females (46%)(Figure 2) with male-to-female (M: F) ratio of 1.1: 1. This male predominance observed in this study has been reported by many other similar studies.^{4,5,7-9} The most common presenting complaint in this study was generalized weakness & pallor (100%) (Figure 3) followed by dyspnea (41%) comparable to other

studies.^{4,5,7}

The most common cause of pancytopenia varied in different studies. In this study megaloblastic anemia was the most frequent (32%) cause of pancytopenia.(Table 1) Same results were seen in various other Indian studies suggesting that nutritional anemia is the most leading cause of pancytopenia in India.(Table 2)

Megaloblastic anaemia can be caused by deficiency of vitamin B₁₂ or folic acid in diet and severity of anaemia depends on ineffective erythropoiesis.¹⁰ In this study, (Table 3) 46 out of 49 cases of it were diagnosed by both BMA and BMB. Among rest 3 cases, 2 were diagnosed as reactive bone marrow and one was of tuberculosis diagnosed

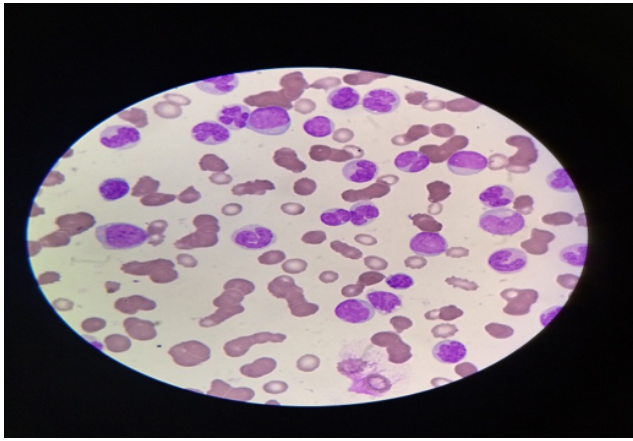


Fig. 5: Bone marrow aspiration smear of acute myeloid leukemia (100x, Field stain)

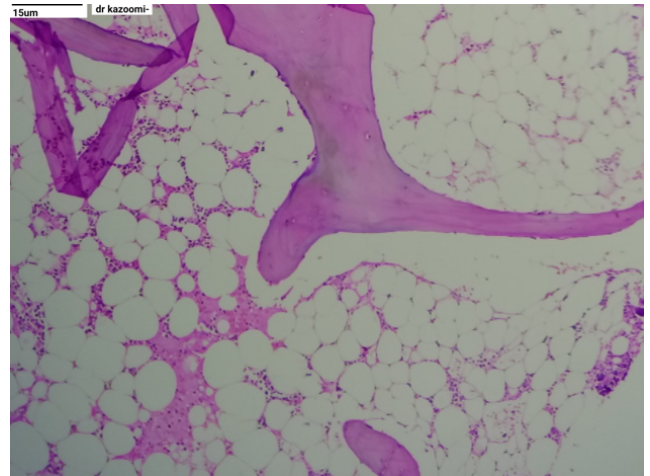


Fig. 8: Bone marrow biopsy of aplastic anemia (10x, H&Estain)

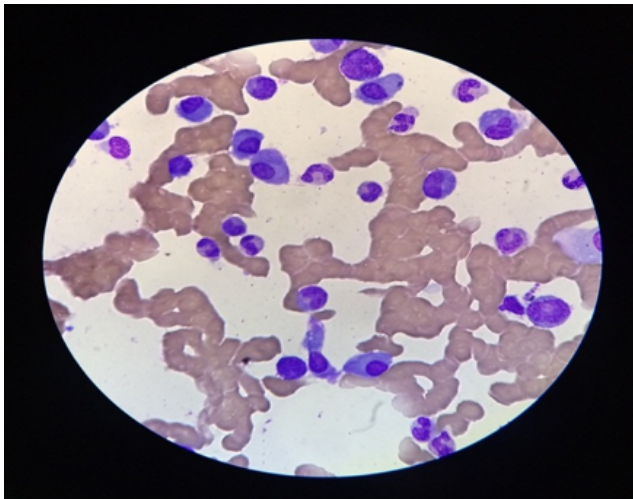


Fig. 6: Bone marrow aspiration smear of plasma cell myeloma (100x, Field stain)

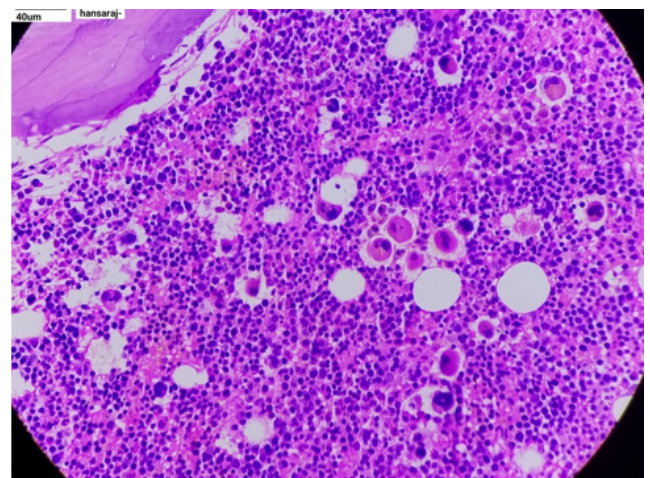


Fig. 9: Bone marrow biopsy of metastasis of adenocarcinoma from gall bladder.(40x, H&Estain)

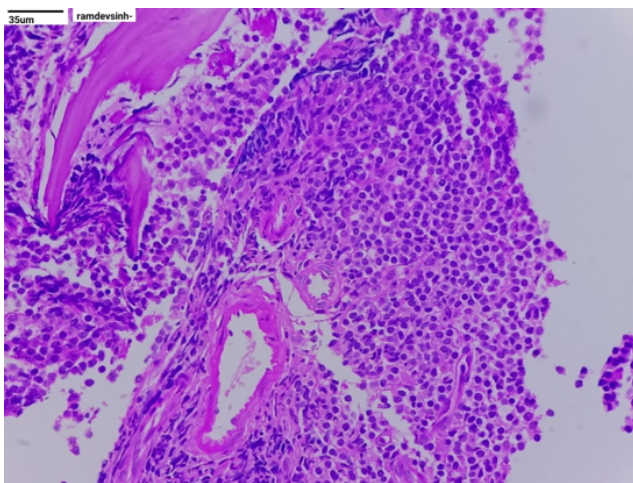


Fig. 7: Bone marrow biopsy of low grade Nonhodgkins lymphoma (40x, H&Estain)

later by BMB. There were mild megaloblastic changes in reactive marrow leading to false positive diagnosis on BMA. In tuberculosis, granulomas were focal and detected on BMB leading to false negative diagnosis on BMA.

Hypoplastic marrow was second most common (33cases) cause for pancytopenia in this study (Table 1) comparable to different Indian studies.(Table 2). The bone marrow were hypocellular due to aplasia, drugs, chronic viral infections, radiation, toxins and hematological malignancies.¹ Aplastic anaemia marrow was hypocellular with relative increase in fat but without abnormal infiltrate or increased fibrosis.(Figure 8) 25 cases were reported same as hypoplastic anemia on BMA and BMB.(Table 3) The rest 8 cases were diagnosed as aplastic anemia(4), metastatic adenocarcinomas(3), reactive marrow (1).

Acute leukaemia-the authors noted 12 cases of acute leukemia both in children and adults in present study (Table 1). In older adults, Acute Myeloid Leukaemia

was more common while acute lymphoblastic leukaemia in childhood. Peripheral blood examination showed pancytopenia with few blasts but diagnosis was established on bone marrow smear showing >20 % blasts. The findings were similar to that detected by Das R⁷ et al, Pereira ADS¹¹ et al, Pathak R¹² et al. On aspiration, dry tap &/or inadequate aspirate (3 cases) were causes for false negative diagnosis. Rest 9 cases were diagnosed by both procedures & thus showing complimentary role to each other. (Table 3) Plasma cell myeloma –Hypo and/or hypercellular marrow was seen with increased lymphocytes and atypical plasma cells. There were 6 cases (3%), (Table 1) which is in the range (1-5.5%) reported by other authors.^{6,7,9,12,13} Lymphoma-Three patients in this study presented with lymphoid neoplasia with pancytopenia.(Table 1) Amongst them, one presented with inadequate material on aspiration and diagnosis was made on BMB revealing hypercellular marrow with diffuse effacement by atypical medium sized lymphoid cells in all cases(Figure 7). These findings were comparable to other studies.^{6,14,15} Thus examination of the BMB is important in the diagnosis and staging of lymphoma.

Metastasis-In present study, 5 cases were detected as metastatic adenocarcinomas on BMB with 2 cases of primary in gastrointestinal tract and 3 cases of prostate carcinoma.(Figure 9) Two of the cases were diagnosed on aspiration smears and three cases were diagnosed by biopsy where aspiration was hypocellular and diluted with peripheral blood(Table 3). 3% metastatic incidence in present study was very well correlated with Shah P⁸ et al and Bahal N⁹ et al studies.

Myelodysplastic syndrome (MDS) is defined as cytopenia in peripheral blood with dysplasia in 1 or more cell lineages.³Three cases of MDS were detected in present study (Table 1) comparable to Pereira ADS¹¹ et al and Sweta¹³et al studies. They were diagnosed as refractory cytopenia with dysplasia in peripheral blood. Bone marrow aspiration showed less than 5% blasts with dysplasia in all the three cell lines.

Myelofibrosis Total 4 cases were diagnosed (Table 3). Out of 4, 2 cases were detected by both BMA and BMB procedures. Rest two cases were showing inadequate material and dry tap on aspiration but BMB supported diagnosis. Bone marrow biopsy showed megakaryocytic proliferation with atypia and reticulin and/or collagen fibrosis grades 2 or 3on reticulin staining. In this study, two patients have given significant drug history for rheumatoid arthritis treatment (secondary causes). These results were comparable to Bahal N⁹et al, Desalphine M¹⁵et al and Jawalgi A¹⁶ et al.

Hypersplenism-there is peripheral pooling of cells with destruction in enlarged spleen occurs resulting in pancytopenia and splenomegaly. Causes are congestive splenomegaly (cirrhosis, congestive heart failure), malaria, leishmaniasis, thalassaemia and hodgkin's disease.¹Present

study had two cases of congestive splenomegaly (Table 1) presented with pancytopenia comparable to other studies.^{11,16}

Tuberculosis is common in developing countries like India and disseminated miliary tuberculosis can cause pancytopenia. In this study, one case of tuberculosis was missed on BMA & later granulomas were detected by biopsy showing complimentary role of both procedures to detect focal lesions.(Table 3)

5. Conclusion

The causes of pancytopenia are diverse and diagnosis of cause is a challenge. Importance must be given to personal, family, nutritional, drugs and alcohol history. Megaloblastic anaemia is leading cause of pancytopenia in India followed by hypoplastic anaemia. Bone marrow aspiration is easy, safe and minimal invasive out patient procedure for evaluation of causes of pancytopenia. BMA and BMB are complimentary to each other if done simultaneously.

The final interpretation is based on the integration of peripheral smear, BMA and BMB study. Clinical features of anemia are observed at the time of detection of pancytopenia. Mean age is approximately 46years with male predilection. Vegetarian population in India should be supplemented with regular intake of cyanocobalamine and folic acid to avoid occurrence of megaloblastic anemia.

6. Acknowledgement

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8. Conflict of Interest

The authors declare they have no conflict of interest.

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