Volume-6, Issue-1, January - 2017 • ISSN No 2277 - 8160 IF : 3.62 | IC Value 80.26

Original Research Paper Pathology

BONE MARROW ASPIRATION AND BIOPSY INTERPRETATION IN ADULT CASES OF PANCYTOPENIA

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ABSTRACT

***BACKGROUND**: Pancytopenia is a relatively common hematological entity. It is not a disease entity but a triad of findings that may result from various disease processes, primarily or secondarily involving the bone marrow. Bone marrow aspiration and biopsy evaluation along with good clinical correlation is of utmost importance to evaluate the causes of pancytopenia and planning further investigations.*

***AIMS AND OBJECTIVES:** Our main aim was to analyse the causes of pancytopenia by evaluating bone marrow aspiration and biopsy and also to correlate the aspiration and biopsy findings.*

***MATERIALS AND METHODS**: The present study is a prospective clinico-hematological study undertaken in M.R. Medical College, Kalaburagi, from November 2014 to October 2015 on hundred patients of pancytopenia fulfilling the inclusion criteria i.e. haemoglobin < 10 gm/dL, total leucocyte count 4.0 × 10^3/μL and platelet count <1.0×10^3/μL. Bone marrow aspiration and biopsy were performed simultaneously in all cases. Perl's stain was done in all cases and special stains like PAS and reticulin were also done wherever necessary.*

***RESULTS AND CONCLUSIONS**: The maximum cases of pancytopenia were in the age group of 46 to 55 years with male preponderance. Megaloblastic anaemia was found to be the most common aetiology of pancytopenia followed by normoblastic erythroid hyperplasia, aplastic anaemias, acute leukemias, myelofibrosis and metastasis. It was concluded from the study that although the advantages of bone marrow aspiration and biopsy differ, both are complimentary to each other and should be performed simultaneously for a complete bone marrow work up and evaluation. It is only through the correlation of clinical, haematological and bone marrow examination findings that proper evaluation and management of patients of pancytopenia can be made.*

KEYWORDS : Bone Marrow, Aspiration, Marrow, Pancytopenia

INTRODUCTION

Bone marrow examination is an established diagnostic modality in the evaluation of various haematological disorders and is extremely helpful in evaluating pancytopenia.[1]

Pancytopenia is reduction in all the three major cellular elements of blood; hence it is the simultaneous presence of anaemia, leucopenia and thrombocytopenia. It is not a disease entity but a triad of findings that may result from various disease processes, primarily or secondarily.[2]

Bone marrow aspiration biopsies are carried out principally to permit cytological assessment but also for immuno-phenotypic, cytogenetic, molecular genetic, and other specialised investigations.[3] This is particularly needed in case of hypoplasia/aplasia and to exclude leukaemia or other malignant infiltration.[4]

Routine aspiration smears may have to be combined with trephine biopsies as quite often aspiration might yield dry or bloody tap. Some experts suggest that marrow examination is essential to the diagnosis, but it has not been established whether the procedure is necessary in all pancytopenic patients.[5]

The present study was undertaken to evaluate the role of bone marrow aspiration and bone marrow biopsy in cases of pancytopenia.

MATERIALS AND METHODS

The present study is a prospective clinico-hematological study undertaken in M.R. Medical College, Kalaburagi, from November 2014 to October 2015 on hundred patients of pancytopenia fulfilling the inclusion criteria i.e. haemoglobin < 10 gm/dL, total leucocyte count 4.0 × 10^3/μL and platelet count < 1.0 × 10^3/μL.

Informed consent was taken from all patients prior to enrolment in our study.

A complete workup of the patients preceded the procedure which comprised of relevant history, physical examination findings, BT/CT/INR, haematological investigations including a complete haemogram, peripheral blood smear study and reticulocyte count.

Bone marrow aspiration and biopsy were performed simultaneously in all cases.

The bone marrow aspiration was performed at the posterior superior iliac spine using Salah needle of 18 gauge.

The bone marrow aspirate was evaluated for cellularity of the fragments, M:E ratio, erythropoiesis, myelopoiesis, megakaryopoiesis, lymphocytes and plasma cells, parasites/abnormal cells/granulomas/storage disorders.

The cellularity was assessed by estimating the percentage of hematopoietic cells compared to fat spaces in the bone marrow. According to Metikurke et al, bone marrow biopsy was said to be

hypercellular when cells were more than 75%, normocellular smears had cells between 25%-75%, and smears with less than 25% cellularity were taken as hypocellular.

Bone Marrow Trephine biopsy was done using Jamshidi needle of 11 gauge.

Adequacy of the biopsy was determined by WHO recommended guidelines, being a minimum 1.5 cm length.

Perl's stain for grading of bone marrow iron stores were done in all cases.

Smears with at least seven fragments were graded by the conventional Gale's method and assessed.

Special stains like PAS and reticulin were done wherever necessary. Quantification of reticulin stain was done into five grades. Various causes of pancytopenia were studied based on aspiration and biopsy findings.

Significant parameters like aetiology, age, gender, clinical features, hematological parameters, peripheral blood film findings, bone marrow aspiration and bone marrow biopsy findings in different cases of pancytopenia were compared with various studies published in literature.

RESULTS

A total of 100 bone marrow aspirations and biopsies were performed.

The age of patients included in study ranged from 18 to 66 years (mean 47.4 years). Maximum numbers of case were seen in the age group of 46 to 55 years (34%) and least seen in age group over 66 years (5%). Of the total number of cases, 56% cases were males and 44% cases were females with male to female ratio of 1.3:1.

The most common symptom was fever (66%) and generalized weakness (59%) which was often prolonged for weeks, other symptoms included pallor, easy fatiguability, bruises, epistaxis, malena, petechial hemorrhages, hematuria and joint pains. Various signs of the patients included signs included splenomegaly, hepatomegaly and lymphadenopathy.

A patient having more than one clinical feature were counted in each category., hence the sum was more than the total number of cases in the study.

A peripheral smear preceded all the aspirations and biopsies. Most common finding observed was dimorphic anemia in 63% cases, followed by microcytic hypochromic blood picture in 56% , hemolytic anemia in 34%. Reticulocytosis was seen in 27% cases and macrocytosis in 23%. Leukemias were diagnosed on peripheral smear in 4% cases. (Table 1)

Cellularity of the bone marrow was assessed taking in consideration the age of the patient. 54% patients presented with hypercellular marrow,13% with hypocellular marrow and 33% with normocellular marrow.

Causes for hypocellular marrow in our study were aplastic anemia(11%) and 2% presented with myelofibrosis (dry tap).

All malignancies and metastasis showed (7%) showed hyperellular marrow.

The most common cause of pancytopenia in our study was erythroid hyperplasia with megaloblastic differentiation (52%)(fig 01) followed by erythroid hyperplasia with micronormoblastic differentiation (28%). Other causes included aplastic anaemia (11%), haematological malignancies (4%) and metastasis (3%). Dry tap was observed in 2 cases. (Table 2)

All aspiration smears were subjected to iron stores estimation using Perls' Prussian blue reaction and graded according to conventional Gale's method.62.5% of patients showed increased iron stores, 25% showed normal iron stores and 12.5% showed decreased iron stores. The increased stores could be due to associated chronic malabsorption syndrome or autoimmune disorder. PAS and reticulin stains were done in lymphomas and myelofibromas respectively.

A positive correlation between aspiration and biopsy was found to be 86% in our study with maximum correlation seen in erythroid hyperplasia with megaloblastic differentiation. (Table 3)

DISCUSSION

There is a wide range of disorders that manifest as pancytopenia. The underlying etiology of pancytopenia varies across different geographical regions. The most common cause of pancytopenia in the present study was megaloblastic anemia (52%) followed by micronnormoblastic (28%), aplastic anemia (11%), leukemias (3%). Other less common causes encountered were multiple myeloma (1%) and metastasis (3%).

In a study conducted by Metikurke et al, megaloblastic anemia (39.5%) was the commonest cause of pancytopenia, followed by nutritional anemia (24.1%), aplastic anemia (12.0%), malignant diseases (18.9%), and others (5.4%) which included uncommon causes like malaria and hemophagocytic syndrome.

Tilak and Jain (1998) found megaloblastic anemia (68%) to be the commonest cause of pancytopenia followed by aplastic anaemia (7.7%) [6]. Kumar et al (1999) found hypoplastic anemia (29.5%) to be the commonest cause followed by megaloblastic anemia.[7]

Khodke et al (2000) observed megaloblastic anemia (44%), followed by hypoplastic anemia (14%) as the common causes of pancytopenia.[8] Mobina Ahsan Sodhy (2005) found megaloblastic anemia (35.9%) followed by hypersplenism (16.3%) as the common causes. Jha et al (2008) found hypoplastic bone marrow (29%) followed by megaloblastic anemia (23.64%) as the common cause in their study.[9] The commonest cause of pancytopenia, reported from various studies throughout the world has been aplastic anemia. However, various studies conducted in the Indian Subcontinent show megaloblastic anaemia to be the most common cause of pancytopenia followed by aplastic anaemia. This seems to reflect the higher prevalence of nutritional anemia in Indian subjects.

Most common complaints in our study and other Indian studies were fever followed by weakness and easy fatiguability. Pallor was the most common sign followed by hepatosplenomegaly.

The findings in this study were similar to those found by Naseem et al where fever (51.8%) was the commonest clinical feature.

Pancytopenia is more common in males. The male: female ratio in our study was 1.3:1, which was similar to a study by Naseem et al (2010) where males were more frequently affected with a ratio of 3.2:1.[10]

The non neoplastic conditions like megaloblastic anemia, nutritional anemia and aplastic anemias were more common than the neoplastic conditions like acute leukemia, which is consistent with other studies.

The hematological parameters were usually nonspecific in many cases and showed considerable overlap. In all these cases a peripheral blood film was of paramount importance in pointing towards a diagnosis of megaloblastic anemia or leukemia.

Peripheral blood film showed hypersegmented neutrophils, macroovalocytes, howell-jolly bodies and dimorphic blood picture in most of the studies which corroborated with our study.

Leukemias were diagnosed on peripheral smear in 4% cases.

Bone marrow aspiration and bone marrow biopsy are important diagnostic tools in the diagnosis of various hematological disorders, including pancytopenia. It is well known that both the procedures are complimentary to each other. Toi et al (2010) found a positive correlation in 61.25% of cases when simultaneous BMA and BMB were performed. They found that the highest correlation was seen with reactive marrow and erythroid hyperplasia.[11] In the study by Metikurke et al, there was 75.8% positive correlation between BMA and BMB with highest correlation in cases of megaloblastic anemia and with erythroid hyperplasia. The erythroid series were showing either normoblastic and/or micronormoblastic maturation.

In the present study, there was 86% positive correlation between bone marrow aspiration and biopsy. Highest correlation was seen with megaloblastic anemia and nutritional anaemia.

Usually only a BMA is performed in cases of megaloblastic anemia, but as these cases presented with pancytopenia both the procedures were performed simultaneously.

Many studies done previously showed that BMB is a better procedure for metastatic tumors.[9]

There were 03 cases of metastasis in the present study which were diagnosed on BMB. Other cases which were diagnosed on BMB included multiple myeloma (fig 02) and myelofibrosis.

Imprint smears were performed in 5 cases in the present study. Rarely, the touch imprints of biopsy cores were necessary to establish a diagnosis. Touch preparations yield more diagnostic identification of individual cells than biopsy sections, and are less likely than marrow smears to disrupt syncytial masses or compact clusters of cells. It is felt that ideally an imprint smear should be obtained for the correct morphology of cells, especially if the aspirate is not good.

Twenty patients with pancytopenia and severe iron deficiency anaemia (< Hb-6.5%) showed a micronormoblastic picture on aspiration with erythroid hyperplasia on biopsy and decreased bone marrow iron stores. 12.5% of the total cases showed decreased iron stores. It has been shown in various studies that iron deficiency presenting with pancytopenia is rare and can be made worse with iron replacement therapy due to decline in leucopoiesis and thromboboiesis. 62.5% of patients showed increased iron stores, 25% showed normal iron stores. The increased stores could be due to associated chronic malabsorption syndrome or autoimmune disorder.

Fibrosis was seen in H&E stained specimen and grading with reticulin stain showed Grade 4 fibrosis in two patients.

CONCLUSION

Pancytopenia is a common hematological disorder that we come across in routine practice. There are numerous causes of pancytopenia which include both non neoplastic and neoplastic conditions. Clinical findings and peripheral smear findings provide valuable information in the workup of patients presenting with pancytopenia and also in planning for further investigations.

Bone marrow examination which includes BMA and BMB is an essential pre requisite for its diagnosis. Though the advantage of each procedure differs, both the procedures are complimentary to each other and should be performed simultaneously along with an imprint smear for a complete bone marrow workup and evaluation.

Bone marrow aspiration is used principally for cytological assessment of the marrow and to study cellular morphology.

Biopsies are better at assessing overall cellularity of the bone marrow and architectural features and hence are more valulable in the diagnosis of aplastic anaemia and myelofibrosis.

Megaloblastic anemia was the commonest cause of pancytopenia in the present study. Most other studies have reported aplastic anemia as the commonest cause. This seems to reflect higher prevalence of nutritional anemia in the Indian subjects. Rare causes like malaria should be kept in mind while making a diagnosis.

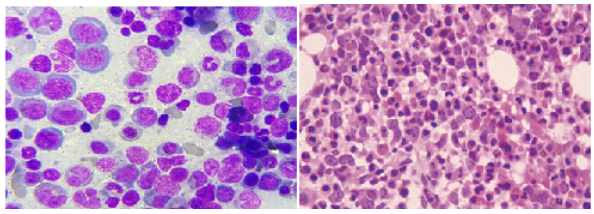

Fig 01 : Erythroid Hyperplasia with megaloblastic differentiation. a) aspirate b) biopsy

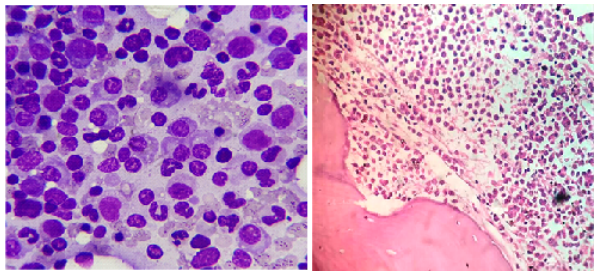

Fig 02 : Multiple Myeloma a) aspirate b) biopsy.

Table No. 01: Peripheral smear findings

	No. of cases	Percentage
Dimorphic anemia	63	63
Microcytic hypochromic anemia	56	56
Haemolytic anemia	34	34
Leukemias	4	4
Reticulocytosis	27	27
Macrocytosis	23	23

Table No. 02: Pancytopenia causes

Cause	Percentage
Erythroid Hyperplasia with megaloblastic differentiation	52
Erythroid Hyperplasia with micronormoblastic differentiation	28
Aplastic anemia	11
Leukemia (subleukemic/aleukemic) ALL AML	 2 1
Multiple Myeloma	1
Dry tap	2
Metastasis	3

Table No. 03: Correlation between aspiration and biopsy

Cause	Aspiration	Biopsy
Erythroid Hyperplasia with megaloblastic differentiation	52	47
Erythroid Hyperplasia with micronormoblastic differentiation	28	23
Aplastic anemia	11	8
Leukemia (subleukemic/aleukemic) ALL AML	 2 1	 2 1
Multiple Myeloma	1	1
Myelofibrosis/Dry tap	2	2
Metastasis	3	3

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