A Comparative Study To Evaluate The Efficacy Of Bone Marrow Aspiration And Bone Marrow Biopsy For Haematological Disorders

Shweta Tevatia¹, Swati J. Patel²*

¹²M.D. Pathology, Pathology Department of Medical College and SSG Hospital, Vadodara, Gujarat.

ABSTRACT

BACKGROUND: To determine utility of Bone marrow examination using Bone marrow aspiration and biopsy in various hematological disorders prior to bone marrow procedure. METHODS: Total 50 cases of various hematological disorders were included in this study. Bone marrow aspiration as well as biopsy was done from posterior superior iliac spine, using Jamshidi-Swaim needle. From bone marrow aspiration crush smear, thin smear were prepared and fixed for 20 minutes with methanol. It was stained with the combination of Leishman and Giemsa stain for 12 minutes. Bone marrow was kept in neutral formal saline for 24 hours, than it was transferred to EDTA solution and kept for 48 hours for decalcification. All the biopsies were routinely processed and stained with Hematoxylin and Eosin staining for light microscopic examination. Prussian blue staining was done on all aspirated smears. RESULT: In present study total 50 patients were included for bone marrow examination with various hematological disorders. Megaloblastic anemia (40%) was the commonest hematological disorder, followed by dimorphic anemia (16%). Patients with Iron deficiency Anemia (3%), Hypoplastic Anemia (3%), sideroblastic anemia (2%), idiopathic thrombocytopenic purpura (6%), hyperplasia (6%), myelodysplastic syndrome (4%), chronic myeloid leukemia (4%), granulocytic sarcoma (2%), idiopathic myelofibrosis (2%) and polycythemia (2%) were also included. Male (n=24) to female (n=26) ratio with various hematological disorders was 0.92:1. Most common hematological abnormality was anemia (98%). Leukemia was present in 48% of cases, pancytopenia in 44% of cases and anisocytosis 74% of cases. Leucopenia, thrombocytopenia, pancytopenia and anisocytosis were more common in patients with megaloblastic anemia. CONCLUSION: Bone marrow examination is justifiable for differentiation of megaloblastic anaemia from macarocytic anaemia. Bone marrow aspiration is superior to Bone marrow biopsy for cytological study. Bone marrow biopsy is superior for determination of cellularity, diagnosis of myelofibrosis and neoplastic disorder over Bone marrow aspiration.

Key words: Bone marrow aspiration, Bone marrow biopsy, Haematological disorders, Anaemia.

INTRODUCTION

Bone marrow (BM) examination is one of the oldest and the most valuable diagnostic tools in the assessment of the haematological disorders. In the initial fetal life haematopoiesis occur predominantly in yolk sac. Some haematopoiesis also occur in liver and spleen. It begins at 1st month of fetal life and ends at 7th to 9th months. Haematopoiesis in the BM starts at 4 months of fetal life and remains same for rest of the life. Distribution of haemopoietic marrow is dependent on age. In the neonate virtually the entire BM cavity is fully occupied by proliferating haemopoietic cells. As the child ages, haemopoietic marrow contracts centripetally, being replaced by fatty marrow. By early adult life haemopoietic marrow is largely confined to the skull, vertebrae, ribs, clavicle, sternum, pelvis and proximal half of humerus and femur bone. BM sample can be obtained by mainly aspiration and trephine biopsy. Proper evaluation of the BM requires clinical history, findings of physical examination and laboratory data. Review of representative blood smear is an integral part of the BM interpretation. Since the first biopsy was performed by Ghedini of Genoa in 1908, numerous reports of BM examination have appeared in the literature discussing various aspects of BM examination, different techniques of BM examination, its utility in different
hematological disorders, role of flow cytometry, cytochemistry, immunocytochemistry, molecular genetics, cytogenetics, electron microscopy and plastic embedding in BM examination. For quantitative assessment of BM differential cell counts of aspirates is being done since decades. It is not usual for similar quantitative assessment of marrow trephine biopsy specimens to be attempted, although there are theoretical advantages in doing so. Aspiration may result in dilution of the marrow by peripheral blood [1-3] and may preferentially sample less adherent marrow cells. BM biopsy should avoid these problems, and cell counts from marrow biopsy could also provide a means of quantifying changes in the spatial arrangement of haemopoietic cells seen in a variety of disease states. Wide array of opinions in the different facets of BM pathology makes it a challenging and interesting topic for study. To determine utility of BM examination using BM aspiration and biopsy in various hematological disorder prior to BM procedure following study was conducted.

MATERIAL & METHODS
Total 50 patients with various hematological disorders admitted in S.S.G. H. (Sir Sayajirao General Hospital) from November 2010 to October 2011 were included in this study. Indication for the BM examination was decided by the clinicians. The clinical history and findings of physical examination were noted. All the necessary investigations including complete blood count, peripheral smear examination, thick smear for malarial parasite, sickling test, reticulocyte count, serum investigations and radiological investigations were carried out before doing BM examination. Indications and complications of BM examination were explained to the patient and written consent was taken. One ampoule (0.5 mg) of atropine was given 15 minutes before procedure. BM aspiration was done from posterior superior iliac spine followed by biopsy using Jamshidi-Swaim needle. The technique employed was the same as that described by Mc Farland et al4. From aspirated marrow particles one crush smear and one thin spread preparation were prepared at bedside. Biopsy core was put in neutral formal saline .In the laboratory particle crush smears and thin spread smears were prepared from the aspirate collected in citrate bulb and fixed for 20 minutes with methanol and stained with the combination of Leishman and Giemsa stain for 12 minutes. After 24 hours biopsy core was transferred from neutral formal saline to EDTA solution and kept for 48 hours for decalcification. All the biopsies were routinely processed .The sections were stained with Harris's Haematoxylin and Eosin for light microscopic examination. Prussian blue staining of aspirated smears of all cases was done. Aspirate smears and biopsy sections were examined microscopically.

RESULTS
The present study included BM examination of 50 cases with various hematological disorders. In the present study Megaloblastic anemia (20 cases) was the commonest hematological disorder accounting for 40% of total study population, followed by dimorphic anemia (8 cases) accounting for 16%. Male (n=24) to female (n=26) ratio with various hematological disorders was 0.92: 1. In both gender megaloblastic anemia was the commonest hematological disorder. Most common presenting complaint was of weakness (n=45), followed by breathlessness (n=25) and fever (n=24). In idiopathic thrombocytopenic purpura bleeding (n=9) was the most common presenting feature and fever (n=24) in hypersplenism. Periorbital odema (n=1) was the only complaint in Granulocytic sarcoma. Pallor was the most common physical finding accounting for 90% of cases. Splenomegaly was present in 38% of cases and hepatomegaly in 30% of cases. Most common hematological abnormality was anemia (98%). Leukopenia was present in 48% of cases, pancytopenia in 44% of cases and anisocytosis 74% of cases. Leucopenia, thrombocytopenia, pancytopenia and anisocytosis were more common in patients with megaloblastic anemia. In 20
cases (40%) of Megaloblastic anaemia BM aspirations were available in all cases and biopsy in 19 cases. BM aspirates showed hypercellular marrow particles with decreased fat cells, pronounced megaloblastic erythroid hyperplasia causing reversal of M: E Myeloid:Erythroid (M:E) ratio, increased mitotic activity with abnormal forms of mitotic figures. Howell - Jolly bodies were also present in some cases. Nuclear chromatin particulated with increase in the parachromatin spaces was also seen. Metamyelocytes with "C" shape or sigma shape nuclei were noted in some cases. Nuclear abnormalities in megaloblasts, particularly particulate nuclear chromatin, were more pronounced in particle crush smear than in thin spread smear. All the 19 BM biopsies showed hypercellular marrow with erythroid hyperplasia except in one case which was normocellular. It was not possible to differentiate megaloblastic hyperplasia from normoblastic hyperplasia on biopsies except in very few cases with severe megaloblastic anemia. In 3 cases (6%) of iron deficiency anaemia BM aspirates showed micronormoblastic erythroid hyperplasia and on the BM biopsies cellularity were normal to mildly increased in all the cases except in one case which showed normocellular areas. In 8 cases (16%) of dimorphic anaemia, BM aspirate smears showed micronormoblastic and megaloblastic erythroid hyperplasia and BM biopsy showed erythroid hyperplasia with hypercellular marrow with decreased iron stores in all the cases. In 3 cases (6%) of hypoplastic anaemia, BM aspirate and biopsy finding were scantily cellular with increase fat space. In one case (2%) of sideroblastic anaemia, BM aspirate showed erythroid hyperplasia with megaloblastic change with 20% ring sideroblast in iron stain and BM biopsy showed megaloblastic change. In 3 cases (6%) of ITP (Idiopathic thrombocytopenic purpura), BM aspirate were hypercellular with markedly increased number of megakaryocytes and predominance of hypolobulated and hypogranular forms cells. BM biopsy showed hypercellularity with increased megakaryocyte cells. Out of 3 cases (6%) of hypersplenism 2 had pancytopenia and one case had bicytopenia. Two cases had normocellular marrow and one had hypercellular marrow. In two cases (4%) of multiple myeloma, BM aspirate was cellular and BM biopsy was hypercellular and showed interstitial sheets infiltrates of plasma cells. In 2 cases (4%) of CML (chronic myeloid leukemia), BM aspirate smear and BM biopsy sections were hypercellular and showed pronounced myeloid hyperplasia. In one case (2%) of Idiopathic myelofibrosis, BM aspirate was dry tap and biopsy section showed diffuse fibrosis. In one case (2%) of Polycythemia, BM aspirate and biopsy showed all three series of hyperplasia with clustering of megakaryocytes with decreased iron stores. Two cases (4%) had normocellular bone marrow. In single case (2%) of extramedullary granulocytic sarcoma, BM aspirate and biopsy revealed hypercellular marrow with no other abnormality.

DISCUSSION

BM examination play a key role in case of diagnostic dilemma like Megaloblastic Anemia, Iron deficiency Anemia, Dimorphic anaemia, Hypoplastic Anemia, Sideroblastic Anemia,ITP, Multiple Myeloma, Chronic Myeloid Leukemia, Granulocytic sarcoma, Idiopathic Myelofibrosis, etc. We have attempted this study to decide the comparative value of BM aspiration and trephine biopsy and its utility in various hematological disorders. Megaloblastic anemia (40%) was the most common diagnosis in present study followed by dimorphic anemia (16 %). Anemia was noted in 98% of patients, in which 76 % of cases had hemoglobin <7.0 gm%. Total 48% patients had Neutropenia, 44% had pancytopenia and 74% anisocytosis. Leucopenia, thrombocytopenia, pancytopenia and anisocytosis were more common in megaloblastic anemia. These results were comparable with Sabharwal et al5 and Gayathri et al6 study. In the present study 44% of patients had Pancytopenia which is comparable to Tilak et al7 study. Severe anemia was present in all the cases while...
in study of Saina et al, it was present in 67%. In patients with megaloblastic anemia BM aspiration showed hypercellular marrow particles with decreased fat cells with pronounced megaloblastic erythroid hyperplasia causing reversal of M: E ratio. It was found that the characteristic nuclear abnormalities in particulate nuclear chromatin were more pronounced in particle crush smear than in thin spread smear. These findings are comparable to that reported by Mallarme et al. It was not possible to differentiate megaloblastic hyperplasia from normoblastic hyperplasia on biopsies except in very few cases with severe megaloblastic anemia. Total 6% of patients had IDA (iron deficiency anemia). BM examination showed micronormoblastic erythroid hyperplasia. Average age of cases of iron deficiency anemia in the present study was 15 yrs, which is lower than in study by Mohammad I et al. According to the study of Mukhopadhyay D. et al BM aspiration and staining for iron provide a definite diagnosis of iron deficiency anaemia or sideroblastic anaemia. It is considered standard for assessing iron status. In the present study 16% patients had dimorphic anaemia. Peripheral smear showed dimorphic picture with microcytes, macrocytes and anisopoikilocytosis increased RDW (Red cell distribution width). BM aspirates Smears showed micronormoblastic and megaloblastic erythroid hyperplasia and BM biopsy showed erythroid hyperplasia with hypercellular marrow. These examination finding were comparable with the study of Zuberi et al. There were 3 cases of hypoplastic anaemia and the average age were 22 yrs which is comparable with the study of Khodke et al which were 26yrs. Aspirate and biopsy were scantily cellular with increase fat space. In the present study hypocellular BM aspirate and biopsy without remarkable pathology is considered as aplastic anemia. There was a single case of sideroblastic anaemia in present study. BM aspirate showed erythroid hyperplasia with megaloblastic change in all series and BM biopsy showed megaloblastic change with 20% ring sideroblast. Ruth et al study on thrombocytosis found that normal and increased platelet are less likely to be associated with leukemic transformation than thrombocytopenia, which was seen in one patient in present study. There were 3 cases of ITP in the present study and they presented with history of bleeding and fever. Platelet count was <1 lac/µl. BM aspirate smears were hypercellular with markedly increased number of megakaryocytes in hypolobulated and hypogranular forms. Biopsy was hypercellular with increased megakaryocyte. According to George et al., in the patients of isolated thrombocytopenia with an otherwise normal hemogram and without any evidence of an underlying disorder by clinical evaluation, a presumptive diagnosis of immune thrombocytopenic purpura can generally be made without a BM examination. In the present study 6% cases had hypersplenism with severe anemia and pancytopenia. BM aspirate showed erythroid hyperplasia. BM biopsies were hypercellular in one case and normocellular in two cases but there was no abnormality in any of cell lineages. Hess C et al described 'primary splenic neutropenia' as a disorder characterized by leucopenia, fever and splenic enlargement. These symptoms were relieved by splenectomy. They recommended that BM examination should be done to rule out a primary blood dyscrasia or other conditions producing leucopenia. They found normal or moderately hypercellular marrow in this disorder. All our cases had massive splenomegaly and there was no cause for hypersplenism in two cases, so primary hypersplenism was the most likely diagnosis. There were 2 cases of multiple myeloma in the present study. BM aspirate were richly cellular. They showed predominance of plasma cells (>30%). Cells of erythroid, and myeloid series were lesser in amount and cytologically normal. Greipp et al defined criteria for myeloma cell typing and classified myeloma based on enumeration of plasmablasts.
immature cells, intermediate cells and mature cells on BM aspiration examination. Majority of the plasma cells in these cases were of intermediate type. Immature myeloma cells and mature myeloma cells were few in number. Plasmablasts were very rare. BM biopsies were hypercellular, showing predominantly interstitial and paratubular infiltrate of myeloma cells. Late normoblasts and megakaryocytes were seen. In Bartl et al study histology and immunohistological parameter were more reliable than cytology in distinguishing reactive from neoplastic plasmacytosis. Plasma cell maturity and BM infiltration predicted survival of the patients and classified as low grade plasmacytic and high grade plasmoblastic type. In present study they were of low grade type. In the present study there were two cases of chronic myeloid leukemia. BM aspirate were hypercellular with myeloid hyperplasia. Erythroid precursors were decreased. Megakaryocytes were unremarkable. BM biopsy was hypercellular and myeloid hyperplasia with left shift and mild megakaryocyte hyperplasia. There were focal areas of fibrosis. That was comparable with study of Gralnick et al. In a series of 143 patients of Chronic myeloid leukaemia, Burkhardt et al found granulocytic predominance in 46% of patients, while mixed granulocytic megakaryocytic variety was found in 54% patients. In the present study there was granulocytic predominance. These finding suggest that our patient had chronic granulocytic leukaemia but not chronic megakaryocytic granulocytic myelosis. In the present study there was a single case of Granulocytic Sarcoma. BM aspirate and biopsy revealed hypercellular marrow with no other abnormality. Periortibital biopsy shows blast cell with eosinophil precursors. On IHC Ckit positive, MPO positive. This finding was comparable with study of Samras et al. In the present study there was a single case of megakaryofibrosis. Splenomegaly was present in the studies of Abu-hilal M et al and Lohman et al was similar in the present study. Hemoglobin value of our case was on the lower side, however WBC count was 48,000 which was higher than that of Abu hilal M et al study, which was 8800, but it was comparable to that of Lohman et al study which was 50,000. Platelet count was 18,000 in present study, which is comparable to that of Lohman et al study. BM aspirate was dry tap. BM biopsy showed diffuse fibrosis with some residual hemopoietic tissue. Megakaryocytes were pleomorphic with hyperchromatic nuclei. Marked sinusoidal dilatation was evident. Pitcoch et al found diffuse fibrosis in all the 70 cases in their study, which was comparable with present study. In the present study there was a single case of polycythemia. BM aspirate and biopsy showed all three series of hyperplasia with clustering of megakaryocytes with decreased iron stores. As per Brain J. et al study, polycythemia can occur in all age groups with mean age of 60 years and patient present with Gastro intestinal disturbance, pruritus and non specific symptoms. In present study patient age was 28 years, with clinical features of plethora, bleeding per rectum, fever and dyspnoea. In present study cases had raised Haematocrit, RBC mass and Haemoglobin without splenomegaly. In the present study there were two cases of normocellular marrow. In one case BM examination was done to find out Kala Azar infection but BM was normocellular and no parasite found. In another case peripheral smear showed prominent macrocytes but BM was normocellular. Patient had history of daily alcohol intake. Alcoholism explains macrocytes in such patients. CONCLUSIONS BM examination is justifiable for the differentiation of megaloblastic anemia from other causes of macrocytic anemia. Whenever clinical and laboratory data do not differentiate iron deficiency anemia from anemia of chronic disorders, BM examination should be done. BM aspirates should be done for detection of micronormoblasts as well as sideroblasts and BM biopsies should be done for determination of cellularity. BM aspirate
smears are superior to biopsy sections for cytologic study and iron study. Particle crush preparation is superior to thin spread smear for the diagnosis of megaloblastic. Differential count in thin spread smear is more accurate than particle crush preparation. BM aspirate, BM biopsy and Peripheral smear examination should be done to rule out medullary involvement in Granulocytic sarcoma preceding leukemia. Peripheral smear examination and BM biopsy is sufficient for the diagnosis of idiopathic myelofibrosis. BM examination is not indicated for the differential diagnosis of isolated thrombocytopenia.

REFERENCES
A comparative study to evaluate the efficacy of bone marrow


