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Comparative Retrospective Study of Bone Marrow Aspirate and Trephine Biopsy

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Abstract

Introduction: Bone marrow aspiration (BMA) and core biopsy have an important role in the investigation and diagnosis of hematological as well as non-hematological malignancies and various other diseases. They are also important in the management of these conditions particularly in the follow-up evaluation of patients undergoing chemotherapy, bone marrow transplantation, typing of anaemia, evaluation of pyrexia of unknown origin and infective diseases and other forms of medical treatment. Involvement of marrow by metastatic tumour, have an effect on clinical treatment and prognosis. Similarly involvement of the marrow by granulomatous lesion especially tuberculous granulomas may be easily identified in bone marrow biopsies. Moreover in cases where malignancies are not clinically suspected, bone marrow aspirations and biopsies have been useful in detecting non-hematologic malignancies. When both the procedures are performed simultaneously, they are complementary to each other there is more material to study the morphology and the pattern of distribution of the cells. **(Riley RS,et,al, 2010)**

Types of bone marrow examination

There are two types of examination done to evaluate the bone marrow studies

- Bone marrow aspiration (BMA)
- Bone marrow trephine biopsy (BMTB)

Bone marrow aspiration

Bone marrow aspirations are carried out principally to permit cytological assessment of bone marrow cells. It should be preceded by evaluation of the medical history and clinical features, results of a full blood count, other laboratory tests, and radiological investigations. It should be carried out by trained individuals who are aware of the indications, contraindications, and hazards of the procedure. They should follow a standard operating procedure. The operator should have made an adequate assessment of clinical and haematological features to ensure both that appropriate indications exist and that all relevant tests are performed. For the patient's comfort and safety, the posterior iliac crest is generally the preferred site of aspiration. Films of aspirated marrow and, when appropriate, films of crushed particles should be made and labelled. Once thoroughly dry, films should be fixed and stained. As a minimum, a Romanowsky stain and a Perls' stain are required. A cover slip should be applied. This is essential to ensure that all appropriate tests are performed on the material obtained and to permit an adequate evaluation. It is necessary to know whether the patient is receiving, or has recently been receiving, any medication that may influence the blood count or bone marrow cytology. This includes drugs that may have an adverse effect on the bone marrow and cytokines that have been given to stimulate haematopoiesis**. (B J Bain, 2001)**

Indications for bone marrow aspiration

Investigation of unexplained microcytosis

- Investigation of unexplained anaemia
- Investigation of unexplained thrombocytopenia
- Investigation of pancytopenia (including suspected Aplastic anaemia)
- Investigation of a leucoerythroblastic blood filmand suspected bone marrow infiltration
- Investigation of suspected acute leukemia
- Assessment of remission status after treatment of acute leukemia
- Investigation of suspected MDS or myelodysplastic/ Myeloproliferative disorder
- Investigation of suspected chronic myeloid Leukemia Follow up of chronic myeloid leukemia
- Investigation of suspected myeloproliferative disorder (polycythaemia rubra vera, essentialthrombocythaemia, idiopathic myelofibrosis, or systemic mastocytosis

Materials and Methods

The present retrospective study entitled "Comparative retrospective study of bone marrow aspiration and bone marrow trephine biopsy" was carried out at Department of Lab Medicine, Fortis Escort Heart Institute Okhla Delhi, From October 2011 to April 2012.A total 220 cases out of them 97 cases of Bone Marrow Aspirate (BMA) and 123 cases are of Bone Marrow Aspirate and Bone Marrow Biopsies (BMB) done simultaneously were included in the study. Out of them 79 (35.9%) were female and 141 (64.09%) were male. The age range of patients varied 20 years to 85 years. All relevant data including clinical records concerning, age, sex, cytochemistry immunohistochemistry, cytogenetic, flowcytometry detail and hematological profile taken from laboratory records of the concern department and from Medical records departments of the hospitals. The pathological material for the study were retrieved from laboratory and reviewed in detail. In all the cases bone marrow slide examined and whenever necessary they were restrained.

Criteria for selection of cases

The following investigation protocol formed the basis of screening for bone marrow aspirate and bone marrow trephine biopsies.

Various modalities used in the diagnosis of bone marrow and classification of the diseases is as follows:

Bone marrow examination Hematological profile (complete blood count by sysmex 1800i).

General blood picture.

Cytochemical stain(MPO/PAS/SBB/PERL'S/MGG)

Histochemical stain (such as H & E)

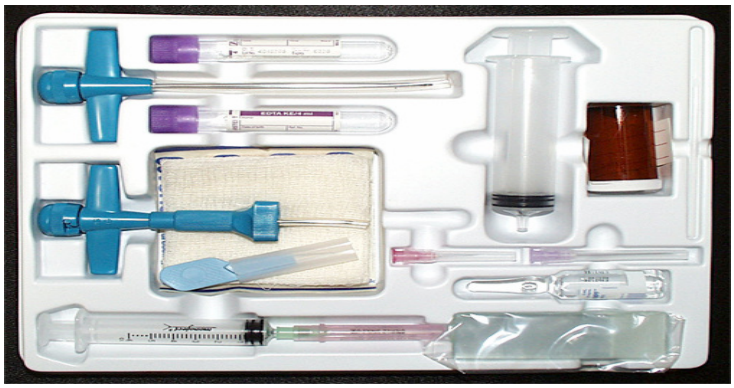


Fig. 1: Commercially available bone marrow procedure kit containing supplies and eauipment for a bone marrow aspirate and biopsy

Site of procedure

Bone marrow aspiration and trephine biopsy are usually performed on the back of the hipbone, or posterior iliac crest. However, an aspirate can also be obtained from the sternum (breastbone). A trephine biopsy should never be performed on the sternum, due to the risk of injury to blood vessels, lun gs or the heart.

Fig. 2: showing site bone marrow

Equipment used for sampling of Bone marrow

1. sterile gloves
2. sterile drape
3. Illinois bone marrow aspiration needle and/or
4. Jamshidi biopsy needle (8-,11- or 13-gauge)
5. Obturator
6. 25-gauge 5/8-inch and 22-gauge 1.5-inch needles
7. number-11 scalpel blade
8. 10-cc Luer slip tip syringes (3)
9. Lidocaine 1%
10. sodium bicarbonate (1 meq/mL)
11. chlorhexadine gluconate 2% or povidone-iodine
12. 4-inch x 4-inch gauze sponges
13. pressure dressing and tape Optional
14. heparinized 10-cc syringe (preservative-free 1000U/mL) for special studies
15. 3.5-inch or 5-inch spinal needle
16. specimen bottle with formalin
17. tube with EDTA anticoagulant

After the procedure

After the procedure is complete, the patient is typically asked to lie flat for 5–10 minutes to provide pressure over the procedure site. After that, assuming no bleeding is observed, the patient can get up and go about their normal activities. Paracetamol (acetaminophen) or other simple analgesics can be used to ease soreness, which is common for 2–3 days after the procedure. Any worsening pain, redness, fever, bleeding or swelling may suggest a complication. Patients are also advised to avoid washing the procedure site for at least 24 hours after the procedure is completed.

Complete blood count (by Sysmax 1800i)

The XT-1800i is a compact, high performance, automated hematology analyzer that provides accurate and precise CBC results including a fully automated WBC 5-part differential.

Table 1 : show different mode working

Sample Aspiration Mode	Measured Sample	Aspiration Volume	Discrete Mode and Throughput		Remarks
			CBC	CBC + DIFF	
Manual Mode	Whole Blood	85 L	80	80	
CP Sampler Mode	Whole Blood	150 L	80	80	Sampler Unit is required.
Closed Mode	Whole Blood	150 L	80	80	It's performed in Sampler Unit.

Preparation of bone marrow slide

Reagents

For smear staining and morphology examination, reagents being used include:

- Methanol solution(for fixation of cells on slide
- Jenner dye(primary stain) (Ranbaxy
- Geimsa dye(counter stain) (Ranbaxy)

Methods

Smear preparation and stain

It is a romonowsky stain, which is basically a mixture of acidic eosin Y(tetra-bromo fluorescein that stain basic groupings if Hb, eosinophil granules), and basic stain of Azur B(Trimethyl thionin, which stain acedic grouping of nucleus, DNA, RNA, neutrophil and basophil granules).

Jenner stain

0.5 percent solution in methanol, i.e., 0.5 gm in 100 ml of methanol, warm to 50 degree Celsius. Then cool to room

temperature and rotate to mix, filter and use. It is the simplest stain.

Geimsa stain

Stock solution: 1.0 gm Geimsa's powder, mix with 62.5 ml of glycerine; keep at 56 degree Celsius for 3 hours. Once it is cool, add 62.5 ml of methanol. This is a complex stain.

Keep staining reagents in dark brown bottles away from light.

Staining procedure

Smears from bone marrow or peripheral blood are prepared and stained as described below:

The smear is properly air dried and fixed by keeping it in methanol for 5 minutes.

Working solution of jenner dye is made and smear is covered with it for 5 minutes.

1. After 5 minutes, Jenner stain is decanted and smear is exposed to workin solution Geimsa dye for 20 minutes.
2. The smear is washed under tap water, air dried properly and labeled.

The primary goal of smear preparation and staining is to study the morphology of cells. This method helps in detecting the lineage of cells present in the specimens of the patients(myeloid or lymphoid), sometimes in case of myeloid, blasts in various stages of development are seen which gives a fair idea of the subtype of myeloid leukemia present. The differential leucocyte count is also performed and blast percentage is noted carefully. Based on these findings these findings the doctor decides further diagnosis.

Special staining techniques used for bone marrow aspirate and bone marrow trephine biopsies are discussed below:

Perl's stain (iron stain):

Periodic Acid Schiff (PAS):

SUDAN BLACK B (SBB):(Ranbaxy)

May-Grunwald-Geimsa stain (MGG):(Ranbaxy)

Hematoxylin and Eosin (H&E) Staining:(Ranbaxy)

Statistical Analysis of Data

The Data was analyzed using SPSS statistical computer programme. The Pearson correlation were applied to show the spectrum of positive correlation between BMA and BMB and used to find the significance between BMA and BMB which were most significant at the level of 0.01(2-tailed). The analysis of variance (ANOVA) was used to compare the mean difference for more than two groups.WINDOW-7 was also used somewhere in the distribution of Age and Sex.

Observation and Results

The present retrospective study entitled "Comparative retrospective study of bone marrow aspiration and bone marrow trephine biopsy" was carried out at Department of Lab Medicine, Fortis Escort Heart Institute Okhla Delhi, From October 2011 to April 2012.A total 220 cases out of them 97 cases of Bone Marrow Aspirate (BMA) and 123 cases are of Bone Marrow Aspirate and Bone Marrow Biopsies (BMB) done simultaneously were included in the study. Out of them 79 (35.9%) were female and 141 (64.09%) were male. The age range of patients varied 20 years to 85 years All relevant data including clinical records concerning, age, sex, cytochemistry immunohistochemistry, cytogenetic, flowcytometry detail and hematological profile taken from laboratory records of the concern department and from Medical records departments of the hospitals. The pathological material for the study were retrieved from laboratory and reviewed in detail. In all the cases bone marrow slide examined and whenever necessary they were restained.

Out of total 220 cases of bone marrow examination, 97(44.09%) cases of BMA done alone and123 (55.9%) cases of BMA and BMB done together. The results for the comparative evaluation were divided into:

- Number of cases where diagnosis was given on BMA alone, BMB was not required

- Number of cases that showed positive correlation between BMA and BMB
- Number of cases where a definite opinion could not be given either in BMA or in BMB.

In present study there was not any cases of BMB alone because at the centre of study BMA was preceded or carried out simultaneously BMB.

Sex distribution on the basis of BMA only

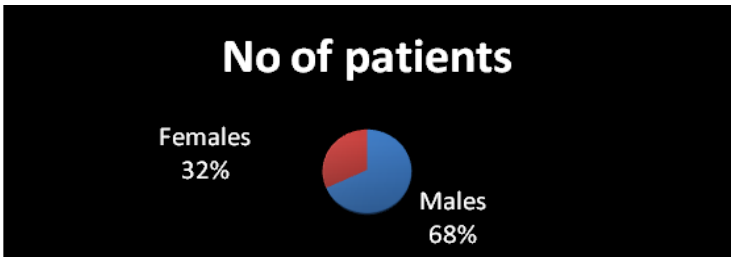
Out of 97 cases of bone marrow aspiration, 66(68.04%) were males and 31(31.95%) as females **(refer to pie-chart no. 5.1 and Table no. 5.1)**

	Males	Females
No. of patients	66	31
Percentage (%)	68.04%	31.95%

Table: 5.1 Sex distribution of patient's were BMA done only

The analysis of data show increase frequency of BMA in Males.

Pie-Chart: 5.1- show sex distribution in BMA studies,



Age distribution of patients with BMA

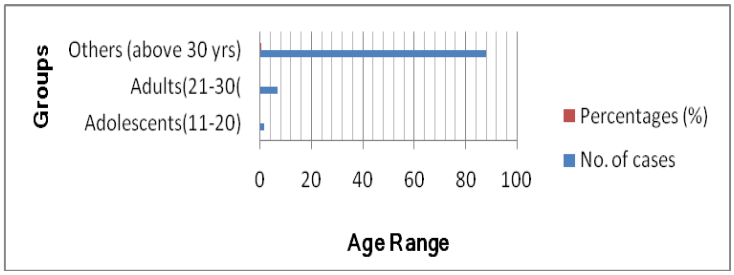
The age of the patient's at time of the diagnosis varied from 20 to 83 years. No age below 20 years was found. Depending on their age differences, they are grouped into three categories. The first group consists of Adolescents within 11-20 years. The second's groups consist of adults within 21-30 years and the third group consists of others above 30 years of age. Majority of the patient's was above the age of 30 years.

Groups	No. of cases(97)	Percentages (%)
Adolescents(11-20)	2	2.1%
Adults(21-30)	7	7.2%
Others (above 30 yrs)	88	90.7%

Table: 5.2 Age distribution of patient's in case of BMA only

In a total of 97 case 2.1% (2) cases were Adolescents, 7.2% (7) cases were adults and 90.7% (88) cases were above 30 years of age, which show highest incidence of BMA diagnosis in patient's above 30 years of age**. (refer to Table no. 5.2 and Bar chart no. 5.1)**

The analysis of data showing increase frequency of patient's above 30 years of age



Bar-Chart: 5.1 Age distribution in case of BMA diagnosis.

Summary and Conclusion

Bone-marrow examination is essential in the investigation of many hematological disorders. It may provide a diagnosis suspected from the clinical features and peripheral blood examination or occasionally gives a previously unsuspected diagnosis. It is also useful in certain diseases for assessing the extent or response to treatment. Bone-marrow fragments may be aspirated and spread on slides, as for a blood film, or a core of bone and marrow may be obtained intact and histological sectioned (trephine biopsy). In general, aspiration is used to show the morphology of individual haemopoietic cells and to obtain material for ancillary tests, whereas a trephine gives a more representative view of the cellularity of the marrow and

allows infiltrations to be recognized. The investigations are limited by the small size of the samples and consequent sampling errors. Infiltrations may be missed, and the cellularity may vary from site to site.

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