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BONE MARROW TREPINE BIOPSY: STILL AN IMPORTANT DIAGNOSTIC TOOL IN THE 21ST CENTURY

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ABSTRACT:
Trepine biopsy is an indispensable diagnostic tool in the 21st century, and despite its long origins, its value remains innovative in developing countries. The outcome of a bone marrow biopsy report depends on many factors, including detailed patient clinical information, cross-departmental affiliations, working knowledge, and the technical capabilities of staff. Molecular studies of biopsies are needed to increase diagnostic accuracy and personalize treatment. We have reviewed articles on trephine biopsy and its challenges to re-emphasize that trepine biopsy are an essential diagnostic tool in our time.
Keywords: Trepine biopsy, diagnostic tool.

Introduction: Although bone drilling is the oldest procedure known to man, the technique was used to diagnose and treat blood disorders only 100 years ago. 10,000 year old skulls showing evidence of medical intervention have been found in Europe, North Africa and Asia, New Guinea, Tahiti and New Zealand.[1] This wide distribution is attributed to Asian ancestry, and many of these "patients" survived, as evidenced by bone healing. Despite its ancient origins, trephine is still used as a suboptimal method in developing countries.[2] However, bone marrow examinations are well known for evaluating various hematologic disorders, no hematologic malignancies, unexplained fever, and infectious diseases. It is true. In the 21st century.[2,3]It can also be used for monitoring patients undergoing chemotherapy and bone marrow transplantation. This procedure can be used to establish or confirm an initial diagnosis of lymphoma or to determine the extent of disease spread for staging.[4]

The technical challenges and diagnostic complexities of bone marrow trepine biopsy (BMT) specimens are poorly recognized. [5,6] Avoiding errors in the histological interpretation of bone marrow trepine biopsy specimens requires some degree of collaboration among histopathologists, hematologists, laboratory scientists, technicians, and assistants. It should be noted that an important starting point is a high-quality sample with complete and relevant clinical information. [5] The causes of errors in the histological interpretation of BMT are: [5,7] Insufficient clinical, hematological, genetic and radiological information. Others were insufficient samples, samples too small or fragmented, poor demineralization/handling, insufficient sectioning (thickness, number of layers), poor staining and inexperienced staff. [6,8,9] Trepine biopsy is a very significant failure in the case of aspiration or dry blow in case of local spinal cord involvement for myelofibrosis, granulomatous lesions, metastatic tumors and lymphomas. [10] Since it has the advantage of being able to perform both aspiration and biopsy at the same time, it is possible to determine the distribution of cells and fat according to the cell type and age of the cells, enabling an accurate diagnosis (Table 3). [11,12]

Indication for Trephine Biopsy: The accepted indications for performing a trephine biopsy includes; inadequate or failed aspirate need for accurate assessment of cellularity, suspected focal lesion and bone marrow fibrosis, need to study bone marrow architecture and blood vessels.[13]These can be divided into absolute and relative indications as shown below.

Table 1: Absolute and Relative Indications of Trephine Biopsy

Absolute Indication
Investigation of suspected Hodgkin's disease and non-Hodgkin's lymphoma
Staging of non-Hodgkin's lymphoma
Diagnosis and follow up of hairy cell leukaemia
Evaluation and follow up of chronic lymphocytic leukemia
Diagnosis of suspected metastatic carcinoma
Diagnosis, staging, and follow up of small cell tumours of childhood
Investigation of suspected myeloproliferative disorders (polycythaemiarubravera, essential thrombocythaemia, idiopathic myelofibrosis, and systemic mastocytosis)
Diagnosis of aplastic anaemia, hypoplasticmyelodysplastic syndromes, and hypoplastic acute myeloid leukaemia
Investigation of an unexplained leucoerythroblastic blood film

Investigation of a fever of unknown origin
Investigation of suspected bone disease
Evaluation of any patient in whom an adequate bone marrow aspirate cannot be obtained
Investigation of patients in whom multiple myeloma is suspected and investigation of selected patients with serum paraproteins without other evidence of multiple myeloma
Relative indications
Investigation of suspected acute myeloid leukaemia
Investigation of suspected myelodysplastic syndrome
Staging of Hodgkin's disease
Evaluation of chronic myeloid (granulocytic) leukaemia
Investigation of suspected primary amyloidosis

Site and Technique of Biopsy

All patients require a written consent if the procedure is to be carried out under general anaesthesia or heavy sedation. Oral consent is sufficient if the patient will be fully conscious during the biopsy but local hospital policy should be followed in this regard.[13, 14, 15] Trephine biopsies should be carried out only by appropriately trained personnel, usually consultant hematologists or haematopathologists or residents in these fields[1,13] The biopsy is done on the posterior superior iliac spine (unilateral), with the patient in the left or right lateral position and with the knees drawn up. An alternative site is the ilium, just below the anterior superior iliac spine, with the patient supine and the approach being perpendicular to the ilium. A *trephine biopsy* should never be performed on the sternum, due to the risk of injury to blood vessels, the heart and lungs.[16] Bone marrow aspiration may also be performed on the tibial site in children up to 2 years of age while spinous process aspiration is frequently done on the L3-L4 vertebrae.[16]Trephine biopsies of the posterior superior iliac spine can be carried out successfully in children. A modified technique applicable to the tibia has been described for neonates.[13]It is preferable to use disposable needles to avoid the risks associated with cleaning reusable needles. Various needle designs are satisfactory used, Jamshidi and Islam needles. Appropriate sterile gloves should be worn and an aseptic technique must be observed.[13]

Local anaesthesia must be adequate with particular attention being paid to infiltrating an adequate area of the periosteum. The adequacy of anaesthesia must be confirmed before proceeding and if technical difficulties are anticipated, sedation is useful. It is not necessary to incise the skin to perform an aspirate but for a trephine biopsy a preliminary skin incision is desirable. The aspiration is usually performed first but, if a very large aspirate is taken, this may lead to disruption of the tissues that are subsequently included in the trephine biopsy specimen, and it is also easier to perform the least painful procedure first.[7,13,15] Bilateral posterior iliac spine trephine biopsies has been found to have advantage over unilateral biopsy in searching for both primary and metastatic malignant neoplasm in the bone marrow and this has been supported by some studies which shows 11-22% increase positivity when the bilateral biopsies were perform.[18]

A core of bone is cut with a hollow needle and the specimen is then aspirated into a syringe; marrow structure is preserved in 10% buffer formalin for at least six hours and the specimen contains small bony spicules that require additional 15minute to 48hoursfor decalcification[16]depending on the nature of the specimen. The use of surface decalcification after processing is sometimes preferred in some centres.

Sample Adequacy: It has been suggested that an adequate trephine biopsy specimen should contain at least five to six intertrabecular spaces and, after processing, should be at least 2-3 cm in length.[13,14] The World Health Organization (WHO)has recommended ≥1.5cm as minimum adequate length[19] (Figure 1) and usually the tissue shrinks by 20% after processing.[16]

Evaluating trephine biopsy specimen

The tissue block is then cut at 2-3µ and at least six sections at 3 levels; 25%, 50% and 75% are prepared.[16] They are then stains with Haematoxylin and Eosin which served as base line for assessing adequacy, pattern and cellulaarity.Giemsa stain help in identifying plasma cells, mast cells, lymphocytes and eosinophils. It also distinguish myeloid from proerythroblast.[16]Other stains include Reticulin, and Periodic Acid Schiff that demonstrates reticulinfibres and glycogen respectively.[16]It is important to have an organized approach not to miss diagnostic features when looking at the stained sections. The specimen should be examined systematically at low power (x4, or x10 objective) to evaluate adequacy, pattern, cellularity, presents of focal lesion, megakaryocytes numbers, osteoclast and osteoblastic activity, (figure 2).At medium power (figure 2) the location of cells of erythroid and granulocytic lineages and their relative proportions can be assessed, the nature of any focal lesions and blood vessels can be examined. Examination at high power is important if fine cellular detail is to be appreciated and if protozoa land fungal infections are to be detected. [1,15,16] Other special stains are requested guided by the disease suspected using the initial histochemical stains.

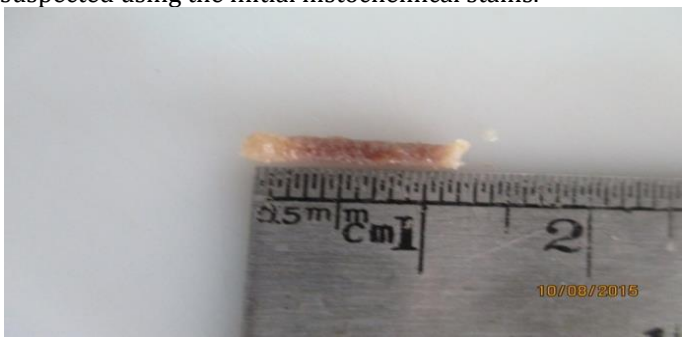


Figure 1: Photograph of gross specimen of trephine biopsy, measuring 1.2cm long, slightly lower than the recommended WHO required length

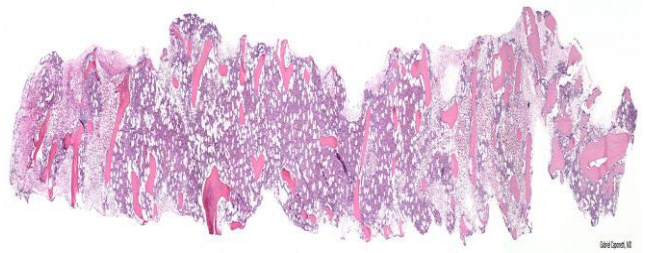
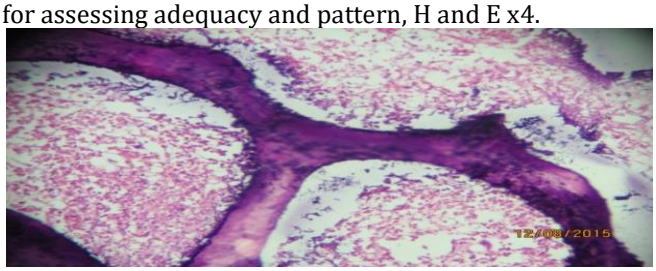


Figure 2: Whole mount trephine biopsy, low power view for assessing adequacy and pattern, H and E x4.



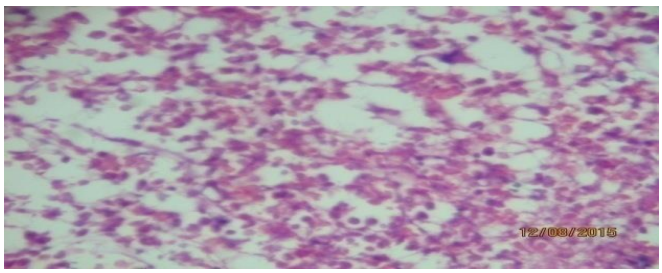
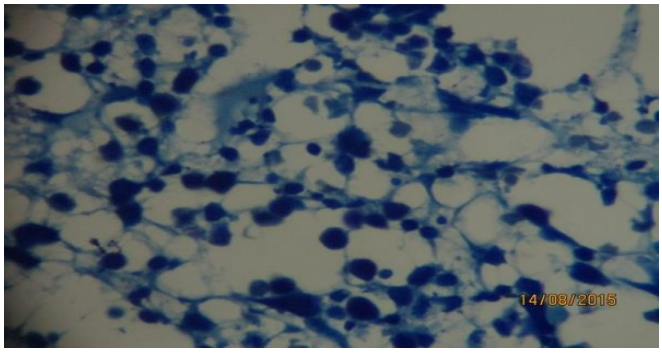


Figure 3: Photomicrograph of bone marrow tissue biopsyshowing marrow cells separated by bony trabeculae, it aid in evaluating pattern and cellularity.H and E x10 and x40



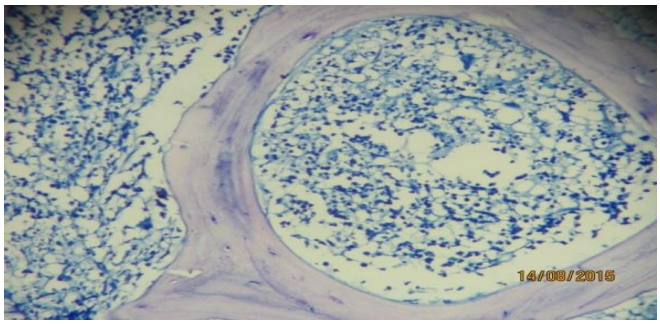


Figure 4: Photomicrograph of trephine biopsy stained with Giemsa, require for details cellular morphology x40 and x10.

Table 2: Age related cell to fat ratio of bone marrow.

Age	Biopsy sites	Cell/Fat ratio
Neonate	All bones, liver, spleen	100/0
Child	Most bones	70/30
Adult	Axial skeleton	50/50
Old age	Axial skeleton	30/70

Table 3: Assessment of bone marrow cellularity and some common pathological disease

CELLULARITY	DISEASE
Hypocellular	Aplastic anaemia,Hairy cell leukemia, Acute myloid leukemia
Normocellular	Be aware of subtle infiltration such as myeloma
Hypercellular	
Homogeneous	Non-Hodgkin lymphoma, Acute leukemia
Heterogeneous	Myeloproliferative syndromes, Myelodysplasias, Metastatic cancer, Small cell tumours of childhood

Table 4: Topography of cellular elements of Bone Marrow

Normal cellular distribution	
Granulocytes	Paratrabeculae and periarterial
Erythroid	Intertrabecular
Megakaryocytes	Intertrabecular and Peri-sinusoidal
Common abnormal patterns	
Myelodysplasia /myeloproliferation	Paratrabecularery throid and megakaryocytic colonies
Follicular lymphoma	Paratrabecular pattern

Table 5: Assessment of cell morphology of bone marrow

Cell morphology	**Disease**
Abnormal megakaryocytes	Myeloproliferation and myelodysplasia
Maturation abnormality	
Maturation arrest	Drug induce
Asynchronous maturation	Myelodysplasia
Abnormal maturation	Megaloblasticanaemia
Imbalance of maturation	Left to Right shift

Reporting trephine biopsy:

The International Council for Standardization in Hematology (ICSH) guideline20 for reporting trephine biopsies is itemized below:

Adequacy and macroscopic appearance of core biopsy

Percentage and pattern of cellularity

Location, number, morphology and pattern of differentiation for erythroid, myeloid, megakaryocytic

Lineages, lymphoid cells, plasma cells and macrophages.

Abnormal cells and/or infiltratesOther findings: Special stains result Immunohistochemistry, when indicated

Florescence in situ hybridization (FISH) and PCR, when indicated

Turnaround time for trephine biopsy is 24-72 hours but additional 1 to 3 days is required if immunohistochemistry or other special stained are performed.[20]External quality assurance for both technical and interpretative elements of bone marrow examination are encourage and recommended to ensure reproducibility and standardization.

Contraindications: There are few contraindications to bone marrow biopsy. It is important to note that thrombocytopenia or bleeding disorders are NOT contraindications as long as the procedure is performed by a skilled clinician.[13,17]

Complications: Mild pain lasting 12 to 24 hours is common after a bone marrow examination, serious complications are extremely rare. In a large review, an estimated 55,000 bone marrow examinations were performed, with only 0.05% adverse effects, including one fatality.[8]In another study in UK out of over 19,000 bone marrow trephine performed in 2003 only 0.08% of total procedures came down with complication mostly due to bleeding.[10,11]

Immunohistochemistry (IHC) –It utilizes antibodies and antibody based technology to detect and localize specific tissue antigens. The basic principle of any IHC procedures is that antibodies will specifically bind with an antigen to produce an exclusive antibody-antigen complex. This bonding is used to visualize both normal and diseased states of tissues. There are many methods for IHC, however, immunnoperoxidase or immune alkaline phosphates are the most common methods that are done manually or by automation. This technology has revolutionized the field of tumors diagnosis and has provided a powerful tool for pathologists to better characterize difficult or unusual neoplasms.[21]Thepanel of antibodies requires depends on suspected tumors diagnosed on H and E and Giemsa stained tissue. These are summarized in the table below.

Conclusion: The importance of trephine biopsy cannot be over emphasized, looking at the emergence of newer technique at both morphological and molecular levels especially in developed countries. Re-examination of the allegory or reality of the test to meet the standard in terms of sample adequacy, ancillary studies and regular inter departmental alliance/conference on the outcome and way forward especially in developing countries is highly recommended and that can aid in accurate diagnosis and targeted therapy.

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