REVIEW ARTICLE

BONE MARROW TREPHINE BIOPSY: STILL A VITAL DIAGNOSTIC TOOL IN THE 21ST CENTURY.

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ABSTRACT

Background: Trephine biopsy is an indispensable diagnostic tool in the 21st century, even though the technique has an ancient origin, its value in developing countries is still innovative. The outcome of the marrow biopsy reports are determined by many factors, which includes detailed patient clinical information, inter departmental alliance, personnel practical knowledge and technical skill. However, molecular studies on the biopsy tissues are highly necessary and that can enhance diagnostic accuracy and also aid in personalized treatment. **Method:** The present review is based on a comprehensive Medline literature search and multidisplinary clinical experiences of the authors'. **Result:** Trepanning is still new in developing countries despite its importance, these has been highlighted especially on molecular ancillary studies and quality. **Conclusion:** We reviewed articles on trephine biopsy and its challenges to reemphasize its usefulness as a fundamental diagnostic tool in this era. Re-examination of the reality of the test to meet the standard is what is required.

Keywords: Trephine biopsy, Diagnostic tool, Overview.

INTRODUCTION

Trepanning of bone is the oldest known procedure carried out by man and yet it is only in the last ten decades that the technique was used to diagnose and care for haematological disorders[1] Skulls dated 10,000 years old showing evidence of medical intervention have been found in Europe, Northern Africa, Asia, New Guinea, Tahiti and New Zealand.[1] This extensive distribution has been attributed to Asiatic origins and many of these 'patients' survived as shown by evidence of healing of their bones. Despite its ancient origin trepanning is sub-optimally utilized in investigation of patients with haematological and non haematological diseases in developing countries.[2] However, is a well known facts that bone marrow examination is an indispensable diagnostic tool in the evaluation of various haematological disorders, non haematological malignancies, pyrexia of unknown origin and infective diseases in the 21st centuary.[2,3] It is also valuable for follow up of patients undergoing chemotherapy and bone marrow transplantation.[4] The procedure serves to establish or confirm a primary diagnosis of lymphoma or to determine the extent of disease dissemination for staging purposes.[4]

The technical challenges and diagnostic complexity of bone marrow trephine biopsy specimens (BMT) are insufficiently appreciated.[5,6] Avoiding errors in the histological interpretation of bone marrow trephine biopsy specimens requires an unprecedented degree of collaboration between histopathologists, haematologists, medical

laboratory scientist, laboratory technical staff and attendants. It should be noted that specimen of good quality, with full, relevant clinical information is the essential starting point.[5] Sources of error in interpreting BMT histology includes;[5,7] inadequate clinical information, haematological, genetic and radiological information. Others are inadequate specimen, too small or too crushed samples, poorly decalcified/processed, inadequate sections (thickness, number of levels), poor staining and personnel insufficient experience.[6,8,9] The trephine biopsy is invaluable in cases where the aspirate fails or is a dry tap as in the case of myelofibrosis, focal marrow involvement as in granulomatous lesions, metastatic tumour and lymphomas.[10] The advantage of doing aspirate and biopsy together enabled the study cellular cytomorphology along with the pattern of distribution of the cells and fats which is age dependent (table 3), and that can help in making the diagnosis accurately.[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 on table 1.

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 haematologists 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 48hours for 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 $\geq$ 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 stained with Haematoxylin and Eosin which serves as base line for assessing adequacy, pattern and cellularity. Giemsa stain helps in identifying plasma cells, mast cells, lymphocytes and eosinophils, sometimes Papanicouolar stains are used. It also distinguishes myeloid from proerythroblast.[16] Chloroacetate esterase is useful for demonstrating myeloid differentiation, although negative in 25% of cases, particularly with immature granulocytic and monocytic neoplasms. Other stains include Reticulin, and Periodic Acid Schiff that demonstrates reticulin fibres 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 details are to be appreciated and if protozoal and fungal infections are to be detected.[1,15,16] Other special stains are requested guided by the disease suspected using the initial histochemical stains.

Reporting trephine biopsy: The International Council for Standardization in Haematology (ICSH) guideline [20] for reporting trephine biopsies are itemized below: (see table 2, 3, 4 and 5)

1. Adequacy and macroscopic appearance of core biopsy (see figure 1)
2. Percentage and pattern of cellularity (figure 2)
3. Location, number, morphology and pattern of differentiation for erythroid, myeloid, megakaryocytic lineages, lymphoid cells, plasma cells and macrophages. (figure 3 and 4)
4. Abnormal cells and/or infiltrates
5. Other findings
6. Special stains result
7. Immunohistochemistry, when indicated
8. 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 stains are performed.[20] External quality assurance for both technical and interpretative elements of bone marrow examination are encouraged and recommended to ensure reproducibility and standardization.

Table 1: Absolute and Relative Indications of Trephine Biopsy

Absolute Indication

1. Investigation of suspected Hodgkin's disease and non-Hodgkin's lymphoma
2. Staging of non-Hodgkin's lymphoma
3. Diagnosis and follow up of hairy cell leukaemia
4. Evaluation and follow up of chronic lymphocytic leukaemia
5. Diagnosis of suspected metastatic carcinoma
6. Diagnosis, staging, and follow up of small cell tumours of childhood
7. Investigation of suspected myeloproliferative disorders (polycythaemia rubra vera, essential thrombocythaemia, idiopathic myelofibrosis, and systemic mastocytosis)
8. Diagnosis of aplastic anaemia, hypoplastic myelodysplastic syndromes, and hypoplastic acute myeloid leukaemia
9. Investigation of an unexplained leucoerythroblastic blood film
10. Investigation of a fever of unknown origin
11. Investigation of suspected bone disease
12. Evaluation of any patient in whom an adequate bone marrow aspirate cannot be obtained
13. 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

1. Investigation of suspected acute myeloid leukaemia
2. Investigation of suspected myelodysplastic syndrome
3. Staging of Hodgkin's disease
4. Evaluation of chronic myeloid (granulocytic) leukaemia
5. Investigation of suspected primary amyloidosis

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	Paratrabecular erythroid 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	Megaloblastic anaemia
Imbalance of maturation	Left to Right shift

Table 6: Bleeding disorders and measures to be taken before trephine biopsy

Disease	**Measure to be taken before trepanning**
Severe thrombocytopenia	Prolong pressure should be applied to achieve primary haemoastesis. Platelet transfusion to raise the count above $15x10^{9}/L$
Coagulation defects,	Correct the coagulation defect before biopsy
Severe liver disease	Correct the coagulation defect before biopsy
Disseminated intravascular coagulation	Correct the coagulation defect before biopsy
Patient who is fully anticoagulated in whom cessation of anticoagulation is contraindicated	Re-assessed the clinical situation and determine an alternative diagnostic procedure which might yield sufficient diagnostic information.

Table 7: *Immuno-histochemical stains* that can be carried out on trephine tissue biopsy.[13,17]

Specific Monoclonal Antibody Or Polyclonal Antiserum	Indications
CD3, CD1a, CD5, CD8	Detection of T cells and T cell subsets (note: CD5 is also expressed on some B cells and B cell neoplasms and CD1a on Langherhan's cells)
CD20, CD79a, CD10, CD23	Detection of B cells and B cell subsets
Terminal deoxynucleotidyl transferase (TdT)	Detection of immature cells in most acute lymphoblastic and some acute myeloid leukaemias
CD34	Detection of immature cells in acute and chronic leukaemias and some cases of myelodysplastic syndrome (also useful for accentuation of vessels)
Myeloperoxidase, neutrophil elastase, CD68	Detection of granulocytic and monocytic differentiation
Glycophorin	Detection of erythroid cells
CD61 or von Willebrand's factor	Detection of megakaryocytes
Light chains (κ or λ)	Detection of isotype restriction in multiple myeloma, MGUS, lymphoplasmacytoid lymphoma, and amyloidosis
CD15	Confirmation of Reed-Sternberg and mononuclear Hodgkin's cells
CD30	Detection of infiltration by anaplastic large cell lymphoma and Reed-Sternberg and mononuclear Hodgkin's cells
CD31	Endothelial cells, macrophages, monocytes, megakaryocytes, and plasma cells
Cyclin D1	Detection of mantle cell lymphoma
ALK1	Detection of anaplastic large cell lymphoma
Epithelial membrane antigen (EMA)	Detection of infiltration by carcinoma cells or anaplastic large cell lymphoma
Cytokeratin, prostate specific antigen, prostatic acid phosphatase	Diagnosis of infiltration by carcinoma cells
CD1a	Diagnosis of Langherhan's cell histiocytosis
Mast cell tryptase	Detection of mast cells and diagnosis of systemic mastocytosis
HMB45 and melan A	Melanoma infiltration of bone marrow

Vimentin, desmin, actin, myoglobin, chromogranin A, protein gene product 9.5, neurone specific enolase	Differential diagnosis of small cell tumours of childhood metastasis to bone marrow
DOG-1,c-KIT,PDGFRA,PKC theta, S100, Desmin, SMA, Myogenin, smoothelin,NSE etc	Soft tissue sarcoma

NB: MGUS, monoclonal gammopathy of undetermined significance.

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

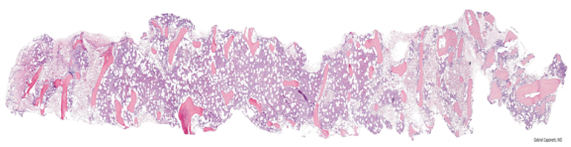

Figure 2: Whole mount trephine biopsy, lower power view for assessing adequacy and patterns, Hand E x4.

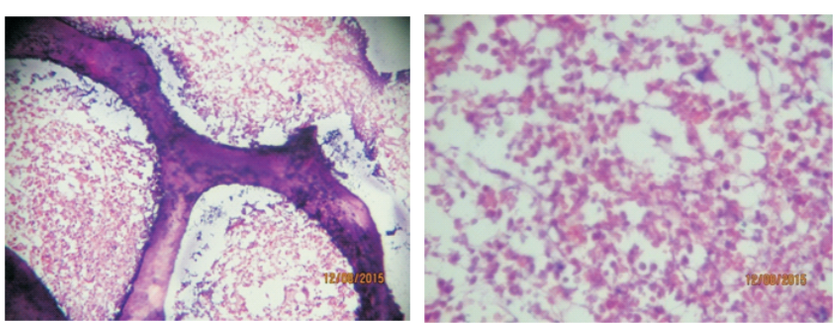

Figure 3: Photomicrograph of bone marrow tissue biopsy showing marrow cells separated by bony trabeculae, it aids in evaluating pattern and cellularity. H and E x10 and x40.

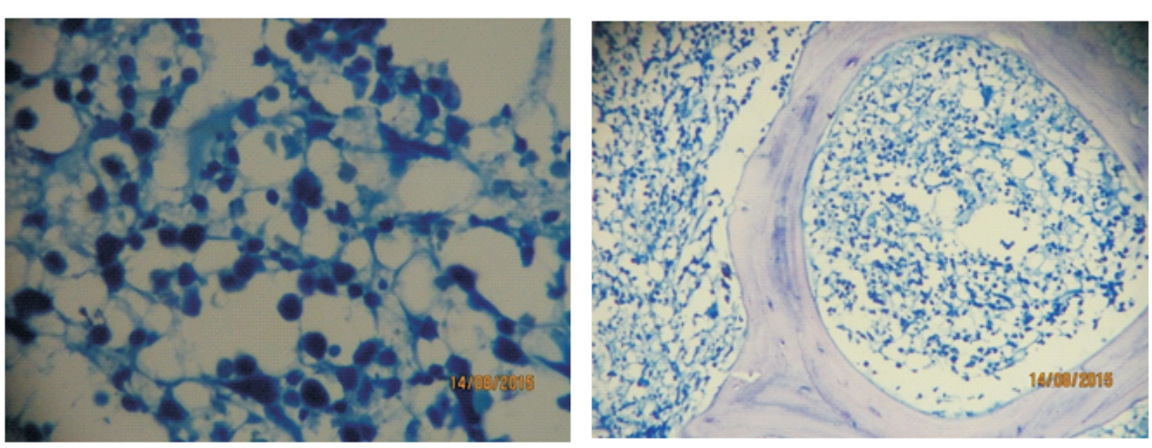

Figure 4: Photomicrograph of trephine biopsy stained with Giemsa, required for detailed cellular morphology x40 and x10.

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] (see table 6)

Complications: Mild pain lasting 12 to 24 hours is common after a bone marrow biopsy, 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 an antibody 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, immunoperoxidase or immunoalkaline phosphotase are the most common methods that are done manually or by automation. This technology has revolutionized the field of tumour diagnosis and has provided a powerful tool for pathologists to better characterize difficult or unusual neoplasms.[21] The panel of antibodies required depends on suspected tumours diagnosed on H and E and Giemsa stained tissue. These are summarized in the table 7.

Conclusion: The importance of trephine biopsy cannot be over emphasized, looking at the emergence of newer techniques 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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