THE ROLE OF TREPHINE NEEDLE BONE MARROW BIOPSY IN THE EVALUATION OF VARIOUS HAEMATOLOGICAL AND NON-HAEMATOLOGICAL DISEASES AT KENYATTA NATIONAL HOSPITAL, NAIROBI.

A dissertation presented in part fulfilment for the degree of Master of Medicine (Pathology) of the University of Nairobi.

By

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April, 1990.

DECLARATION

I certify that this is my original work and has not been presented for a degree in any other University.

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DEDICATION

To Rajab, Rama and Ivy.

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TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

LIST OF APPENDICES

SUMMARY

This is a descriptive retrospective and prospective study of 101 patients admitted to Kenyatta National Hospital (KNH) between 1st October, 1985 and 30th January, 1990 and had bone marrow examination done by aspiration and trephine needle biopsy. At KNH trephine needle bone marrow (TNBM) biopsy has been performed over the years when aspiration results in `dry tap'. It is only available for the use by a few specialists (haematologists) and it is rarely performed in staging malignant lymphomas or as a routine diagnostic technique in various other diseases. The study was done to evaluate the role of the technique in patient care at this hospital. The relevant data and the diagnostic outcome of 50 patients admitted to the hospital between 1st October, 1985 and 30th June, 1989 were collected retrospectively. Trephine needle biopsies were performed by the investigator on 51 patients during the last 7 months of the study. Data collected included: the age and sex, the indications for bone biopsy, the quality of the specimen, the reporting format and the final diagnosis on the trephine biopsies.

The 101 patients studied were between 2 and 75 years of age. The mean age was 23.9 years. There were 62 males and 39 females. A `dry tap' aspirate, the commonest indication for TNBM biopsy was reported in 37 (36.6%) cases. In twelve cases, the aspirate and needle biopsy were performed at the same time using the same needle. Ten of these were in the staging of malignant lymphomas. Good or satisfactory specimens were obtained in at least 86% of the biopsies

performed. Aplastic anaemia, the commonest abnormality detected was found in 28 (27.7%) of the patients studied. A review of the reporting format showed that in only 20% cases in the retrospective study was a full report of the biopsy given by the haematologist.

This study shows that TNBM biopsy is a simple and safe procedure yielding a good or satisfactory specimen in most instances. The biopsy will most likely provide a diagnosis when bone marrow aspirate fails due to `dry tap' or scanty yield. The procedure may be of value in routine investigation of various diseases such as aplastic anaemia and in staging of malignant lymphomas although larger studies need to be done in this area (only ten cases in this study). A standard format should be formulated and adhered to by haematologists and pathologists reporting on the TNBM biopsies in this hospital.

INTRODUCTION

Bone marrow examination has been the cornerstone of haematology practice since its introduction into routine clinical use in the 1940s (1). Many instuments have since been devised to sample bone marrow (1, 2, 3, 4). In 1958, MacFarland and Dameshek developed a simple technique for bone marrow biopsy using the Vim-Silverman needle (5). The Westerman-Jensen needle was introduced shortly thereafter. Ideally the bone marrow biopsy should be easily obtained on the first try with minimal discomfort or risk to the patient. The specimen should be of adequate size without distortion of structure and it should be possible to obtain an aspirate as well as a biopsy through the same instrument. Recently the Jamshidi needle has become commercially available (6). Unlike previous devices which obtained small and frequently crushed specimens, the Jamshidi needle, because of its unique construction, allows for greater patient comfort and safety and at the same time minimizes crushing artefact of the marrow specimen. Additionally, it requires minimal maintenance and has considerable longevity compared to other bone marrow biopsy needles.

The relative diagnostic merits and limitations of bone marrow biopsy are well appreciated. In standard practice, bone marrow is aspirated through a large gauge needle (Klima or Salah) and spicules of marrow are smeared on clean glass slides and examined after appropriate staining. Several

millilitres of marrow can be aspirated and may either be cultured for micro-organisms or may be embedded in paraffin for histologic sections. Such paraffin sections facilitate examination of larger amounts of marrow, but lack the fine morphological detail seen in marrow smears.

Trephine needle bone marrow biopsy has made it possible to obtain a core of bone and its enclosed marrow (6). An aspirate can be obtained through the same biopsy needle prior to performing the marrow biopsy. It has also obviated the need for open surgical biopsy and can be performed on an outpatient basis. The biopsy specimen is generally obtained from the posterior superior iliac spine and in experienced hands, complications are rare and the procedure has minimal morbidity.

Needle biopsy has become the standard method for obtaining a marrow specimen when aspiration leads to a `dry tap'. Moreover, evaluation of bone marrow cellularity and abnormal architectural patterns and detection of structures other than haematopoietic cells within the marrow are best achieved by bone marrow biopsy. The marrow core biopsy is most useful in evaluating diseases that characteristically produce focal rather than diffuse marrow involvement. Transilial trephine needle bone biopsy followed by histomorphometric quantintation is a common procedure for detection and management of metabolic bone diseases (7).

The biopsy in most instances is not a substitute for examining the marrow by aspiration and smear, but it is a complimentary procedure which provides additional information. The aspirate provides cytological detail not readily seen on histology. In a core biopsy, it may not be possible to classify an immature cellular infiltrate as myeloid, lymphoid or even erythroid. Megaloblastic changes are also better appreciated in the aspirate. Until recently most of the supporting cytochemical stains could only be done on the smear (8).

LITERATURE REVIEW

The term marrow cellularity refers to haematopoietic elements (red marrow) which comprises the erythroid, myeloid and megakaryocytic series as well as mixtures of lymphocytes, plasma cells, mast cells and reticulum cells. Yellow marrow refers to mature adipose tissue.

The marrow of the newborn infant is composed almost entirely of red marrow, with virtually no or very few fat cells. As the infant matures, much of the red marrow is replaced until, in the normal adult, red marrow is principally found only in the skull, sternum, clavicles, ribs, scapulae, vertebrae, pelvis and proximal long bones. Thus the degree of marrow cellularity will vary with skeletal location, size of sample and age of patient. For example, the anterior iliac crest has a greater amount of haematopoietic tissue than the rib, but less than the sternum. A bone marrow sample from the anterior iliac crest of a 10 year old contains approximately 80% marrow which decreases to 50% by the age of 30. Thereafter it remains stable until age 70, when the red marrow content decreases to about 30% of the specimen. Cellularity of biopsies taken from the posterior superior iliac spine ranges from 30 to 75% (8).

There may be considerable variation in cellularity within a single specimen. Sample size is extremely important and conclusions about such a large organ as the

bone marrow must be put into proper perspective when drawn from a single biopsy sample.

Krause et al. (8) have performed and examined 15,000 bone marrow biopsy specimens over 21 years. They conclude that bone marrow needle biopsy is of importance when one or more of the following disorders are suspected:

- Disseminated granulomatous disorders;
- Malignant lymphoma;
- Myeloma including amyloidosis;
- Myeloproliferative disorders;
- Aplastic anaemia;
- Metabolic bone disease;
- Storage diseases;
- Alteration in marrow iron stores;
- Clinical staging of Hodgkin's disease and Non-Hodgin's lymphomas.

There are virtually no contraindications to the procedure (9). It has been performed in patients with severe thrombocytopenia and other haemorrhagic disorders without ill effects. The simplicity of the procedure and low patient morbidity has virtually eliminated the need for open surgical biopsy (10, 11, 12).

Besides being the site of origin of numerous primary haematopoietic malignancies, the bone marrow is commonly involved by metastatic tumours. The presence of metastases in the bone marrow usually means an incurable although not necessarily a rapidly fatal disease. It is therefore

important to rule out marrow involvement in any malignancy for which curative treatment is being considered. Metastasis may be present in the marrow without any abnormalities in the bone scans, radiographic pictures, serum chemistry or other haematologic parameters (13). The `dry taps' obtained during aspiration may be related to the tumour-associated fibrosis or osteosclerosis of the marrow, or both, that prevents aspiration of haematopoietic elements (14). Bone marrow biopsy is therefore superior to the aspirate smear in detecting metastatic tumour (15, 16, 17, 18).

In one study (19) examination of bone marrow from patients with cancer revealed presence of metastatic cells in 10 percent of cases. In half of these, marrow involvement was the first evidence of `hidden' or occult malignancy. Metastatic tumour may appear in the marrow as solid sheets, nests or cords of cells. The problem of tumour identification in the bone marrow has previously been noted (20). Over 25 cell types and their variants are normally present in the marrow (20). The tumour cells are generally larger than normal haematopoietic cells, and have a large hyperchromatic nuclei with prominent nucleoli. When the tumour cells resemble the cells in the primary lesion, recognition becomes simple. Gland formation is often evident in metastases from tumours of the prostate, breast or gastrointestinal tract. With modification of plastic embedding techniques, non-morphologic techniques have been developed in aiding the identification of metastatic

malignant neoplasms in bone marrow (7); for example, metastatic prostatic carcinoma may clearly be identified with prostate specific antigen (21). Due to the silent nature of this disease diagnosis is frquently delayed and presence of tumour cells in bone marrow biopsy may be the only evidence of disease beyond the prostate (22).

Depending on the marrow sampling site, metastatic tumour may occupy the entire biopsy or may be found only in a small focus. The commonest reaction to tumour in the marrow is a desmoplastic or fibrotic response. New bone formation and necrosis are also frequently observed (14). The presence, therefore of these reactions even in the absence of clearly identifiable tumour, is highly suggestive of metastatic carcinoma. Apart from the myelofibrotic and osteosclerotic response, areas of the marrow not involved by the tumour or surrounding the tumour may be normocellular, hypercellular or hypocellular. Magakaryotypes are frequently increased and there may be prominent eosinophilia. Unexplained anaemia, thrombocytosis or eosinophilia should prompt physicians to look for metastatic tumour in the marrow.

In staging of patients with a known primary malignancy, radiological bone surveys are more sensitive than marrow biopsies in detecting metastatic disease (23, 24, 25). However, a lytic lesion must be 1 to 1.5 cm in diameter to be visible on the X-ray. Therefore, a significant number of positive biopsy specimens obtained from different sites

increase the diagnostic yield. Unfortunately with the standard bone marrow biopsy needles sampling can only be performed safely from the anterior or posterior iliac crests, or occasionally from the vertebral bodies. Hansen et al. (13) demonstrated the usefulness of bone marrow aspiration and biopsy in evaluating carcinoma of the lung, especially the oat cell type. Although determination of serum acid phosphatase in carcinoma of prostate has been advocated as a reliable indicator of detecting early metastases (26), the significance of an elevated bone marrow acid phosphatase level in the absence of diagnostic bone marrow biopsy, bone scan or radiological series is not clear.

The selection of either supervoltage radiotherapy and/or combination chemotherapy in treatment of Hodgkin's disease requires accurate clinical staging, for treatment depends in part upon stage of the disease. A needle or open biopsy technique is essential for the identification of Hodgkin's disease involving the bone marrow. Marrow aspiration is usually negative or yields inadequate material for interpretation due to `dry taps' (27, 28). This is due to the patchy, fibrous or granulomatous nature of the lesion which prevents aspiration and is in part due to disruption of the marrow architecture by aspiration. Marrow involvement is often the only evidence of stage IV Hodgkin's disease (29) and marrow biopsy rather than aspiration is useful for detecting this as marrow architecture is

preserved and a generous plug of marrow can be easily obtained.

The incidence of bone marrow involvement in Hodgkin's disease varies from 5 to 15% of untreated patients at the time of initial diagnosis (27, 29, 30, 31). The frequency of bone marrow involvement varies according to the histological type. O'Carroll et al (29) found bone marrow involvement in 2 out of 3 patients (67%) with lymphocyte depletion, 6 out of 27 patients (22%) with mixed cellularity, 5 out of 27 patients (8%) with nodular sclerosis, and 2 were unclassified. Hodgkin's disease patients with bone marrow involvement are invariably symptomatic (29). Laboratory parameters are not reliable in predicting bone marrow involvement with Hodgkin's disease (27).

In many cases of Hodgkin's disease, pathologic identification of bone marrow involvement may be difficult since Reed-Sternberg cells may not be evident. In patients with well documented Hodgkin's disease, biopsy specimens showing mixtures of lymphocytes, a anaplastic histiocytes and fibrosis, even in the absence of identifiable Reed-Sternberg cells are regarded as diagnostic by most investigators. As the morphologic characteristics of Hodgkin's disease in the marrow may not be identical to those seen in the lymphnodes, the histopathologic classification should not be inferred from the marrow biopsy. Involvement of bone marrow in Hodgkin's disease is

associated with a relatively short survival hence the need for aggressive combination chemotherapy to produce a significant remission.

Utilizing bone marrow biopsies, Vinciguerra and Silver reported a very high incidence of marrow involvement in non-Hodgkin's malignant lymphomas (ML) (32). A high incidence of bone marrow involvement is found with poorly differentiated lymphocytic types (33). Bilateral bone core biopsies increases the yield of positive specimens obtained from a single iliac crest (34). As with Hodgkin's disease, there are no pretreatment laboratory findings which enable to predict which patients would have a positive bone marrow biopsy. Conversely an entirely normal haemogram does not exclude the presence of marrow invasion (33, 34). Overall frequency of bone marrow involvement with ML ranges from 15 to 63% (35, 36).

The possibility that the bone marrow may be the site of origin of some lymphoid neoplasia cannot be ruled out. The incidence of normal lymphoid nodules in bone marrow varies from 4 to 47% in the older age groups (39). They are found especially in patients with chronic myeloproliferative disorders, inflammatory reactions and haemolytic conditions. Should these nodules undergo hyperplasia, differentiation from malignant lymphomas may not be possible on morphological grounds (40).

From the immunological point of view, B cell lymphomas are more frequently found in the bone marrow than those of the T-cell lineage (37). Hairy cell leukaemia and immunocytomas may be detected in bone marrow when other indications of spread are minimal or absent. The diagnostic criteria and classification for ML on histology, cytology, cytochemical and immunological markers applied to findings in an excised lymphnode can be applied to bone marrow biopsies (38).

Granulomas, whether in the bone marrow or any other organ, represent a host inflammatory response which may be elicited by a wide variety of stimuli, and the granulomatous lesion is notorious for its aetiologic diversity. Unless a specific organism can be identified by special stains or bacteriologic culture, there are no diagnostic features of a granuloma characteristic of a definite disorder although prominent caseous necrosis favours tuberculosis or histoplasmosis (41). Nonetheless, demonstration of the bone marrow granuloma allows for a considerable narrowing of the differential diagnosis. The large sample obtained by bone marrow biopsy and the lack of architectural distortion renders the technique superior to the aspirate for demonstration of granulomas.

Bone marrow biopsy has been shown to be of value in the diagnosis of disseminated tuberculosis, histoplasmosis and sarcoidosis (42, 43, 44). Bone marrow examinations are diagnostic in 15 to 40% of cases of miliary tuberculosis,

especially in the early course of the disease when the initial chest radiographs may be considered normal (43). Granulomatous lesions are also found in the bone marrow in a very high percentage of cases of brucellosis (41, 45) and positive bone marrow cultures have been reported at times when simultaneous blood cultures have been sterile (46).

Needle biopsy of bone marrow has been shown to be superior in the evaluation of stainable iron stores. A significant over diagnosis of iron deficiency may occur if only aspiration smears are evaluated (47, 48). The large histological specimen allows more accurate evaluation of iron stores than do marrow smears.

Single or serial biopsies of bone may be used for calcium metabolic studies and in assessment of toxic elements such as lead which are preferentially concentrated or stored in bone (9).

JUSTIFICATION

In Kenya the technique of TNBM biopsy is currently only available at KNH which acts as a referral and teaching hospital. Indeed the technique is only confined for use to a few specialists. The number of patients in which the technique is performed is therefore very limited. This study was therefore undertaken to determine the role and value of this procedure in diagnosis and management of various conditions at this hospital and if found useful, to try and offer recommendations and guidelines for the indications and use of this procedure in KNH and other hospitals in the country.

OBJECTIVES

General Objective:

This study was designed to describe and verify the usefulness of the trephine needle bone marrow (TNBM) biopsy in diagnosis and management of various diseases at KNH and to seek ways of promoting this technique if it is found to be of advantage.

The specific objectives were:

1. To assess the merits and demerits of the TNBM biopsy in diagnosis or confirmation of various diseases for which the technique is indicated.

2. To show the role of the TNBM biopsy in staging of Hodgkin's and non-Hodgkin's lymphomas.

3. To identify any secondary histopathological changes in bone marrow which may be associated with the disease being investigated.

4. To assess the quality of the TNBM biopsy specimens obtained for analysis.

5. To analyse and review the reporting format of the TNBM biopsies.

MATERIALS AND METHODS

Place of Study and Study Population

The study was carried out in retrospective and prospective phases. The retrospective phase involved 50 patients who had been admitted into KNH between 1st October 1985 and 30th June 1989 and had bone marrow studies done by aspirate and trephine biopsy. In the prospective phase, 51 bone marrow trephine biopsies were performed on patients admitted to the Adult Medical and Surgical Wards, Paediatric Medical, Surgical, Oncology and Emergency Wards at KNH over a period of seven months.

Data Collection:

a. Retrospective Study:

Inclusion Criteria:

The reports of all TNBM biopsies performed between 1st October 1985 and 30th June 1989 were extracted from the Department of human Pathology, Haematology section.

The case files of these patients were retrieved from the Records Department and the following information obtained: the clinical presentation, diagnosis, indication for aspirate and trephine bone biopsy, the reporting format and final diagnosis on examination of the biopsy. This and

other relevant information was extracted onto a proforma (See Appendix I).

Exclusion Criteria:

Patients whose files could not be traced were excluded from the study.

b. **Prospective Study:**

Inclusion Criteria:

1. All cases for which trephine bone marrow biopsy was requested for by the Ward Doctors as part of the patient work-up.

2. Patients with Hodgkin's and non-Hodgkin's lymphoma whose marrow examination had not been done as apart of the staging procedure.

3. Patients in whom bone marrow aspirate had yielded a `dry tap'.

4. Patients with various diseases where diagnosis was obscure. For example patients with pyrexia of undetermined origin, patients with suspected disseminated malignancy where bone marrow examination would aid towards providing a diagnosis.

Exclusion Criteria:

Patients with known bleeding disorders.

Laboratory Methods

a. **Peripheral Blood Examination**

Venous blood collected in Ethylenediamine tetra acetate (EDTA) was processed through Coulter Counter model S plus (IV) and the following indices recorded:

- Haemoglobin (g/dl)
- Packed Cell Volume
- Mean corpuscular haemoglobin concentration (g/dl)
- White Blood Cell Count (WBC)
- Red Cell Count (RBC)
- Platelet Count.

Peripheral blood films were stained by the May Grunwald-Giemsa (MGG) technique (Appendix II) and examined for differential WBC count, red cell and platelet morphology and presence of parasites. The findings were correlated with those of the bone marrow examination.

b. **Bone Marrow Examination**

Bone marrow aspirate and trephine needle bone marrow biopsy were obtained from the posterior superior iliac spine using the Jamshidi needle [Kormed 2nd Generation gauge II length 4" (American Pharmaseal Laboratories California)] (Appendix III). The bone marrow smears were stained by the MGG technique. The trephine biopsy was processed and embedded in paraffin for histological sections. These were

then stained with the standard haematoxylin and eosin stain, Perls Prussian blue for iron and Gordon and Sweets method for reticulin. Other special stains were done as indicated for example Periodic Acid Schiff and Masson Trichrome (Appendix IV) using standard procedures.

The bone marrow smears and biopsy sections were examined by the author under direction of a qualified haematologist and histopathologist.

The following format was used for reporting the trephine biopsy:

QUALITY: recorded as adequate or inadequate. Optimal specimens are approximately 1.5-3.5 cm. in length and have a wet weight of about 150 mg (8). After processing the cores should remain intact, at microscopy showing a core of bone spicules with bone marrow enclosed within. The following grading was used:

GOOD - optimal specimen

FAIR - fragmented but adequate material, enough for a specific diagnosis to be made without difficulty.

POOR - fragmented/Inadequate/poorly processed material. Difficult to assess. Diagnosis if reached with some difficulty.

The same grading was used in the retrospective study as follows:

GOOD - Comment on report as `good core'.

FAIR - Comment on report as `fragmented or small but adequate'.

POOR - Comment on report as `poor', `inadequate' or `poorly processed material difficult to evaluate'.

NO COMMENT - No comment given on the report as per quality of specimen.

CELLULARITY - recorded as hypercellular, normocellular, hypocellular.

HAEMOPOIETIC ACTIVITY - Myeloid:Erythroid ratio, Maturation of myeloid/erythroid series, Megakaryocytes quantity and Morphology.

NON-HAEMOPOIETIC CELLS: Lymphoid cells, plasma cells, mononuclear phagocytic cells, foreign cell infiltrate such as carcinoma cells and abnormal storage cells.

PARASITES: Present or absent

IRON STAIN: Increased, present and normal, or reduced, or absent.

ADDITIONAL ABNORMALITIES: such as stromal abnormalities (granuloma, fibrosis, necrosis, serous atrophy of fat), haemosiderrin content, vessel abnormalities (such as amyloid deposit) and bone changes were noted from the trephine biopsy.

Ethical Consideration:

The study was carried out with the approval of the KNH Research and Ethics Committee (Appendix V). Informed consent was obtained verbally from the patients or their parents or guardians (49).

RESULTS

Fifty patients were studied retrospectively and 51 trephine needle biopsies were performed in the prospective study.

The ages ranged from 2 to 75 years (Figure 1). There were 62 males and 39 females giving a Male : Female ratio of 1.6:1. Forty three (42.6%) patients were in the age group 0-14 years. This is because of the existence of a specialised paediatric oncology ward within KNH and therefore a larger number of requests for trephine biobsy were received from the paediatric wards in the prospective phase of the study.

Figure 1: Histogram showing the age and sex distribution of the 101 patients.

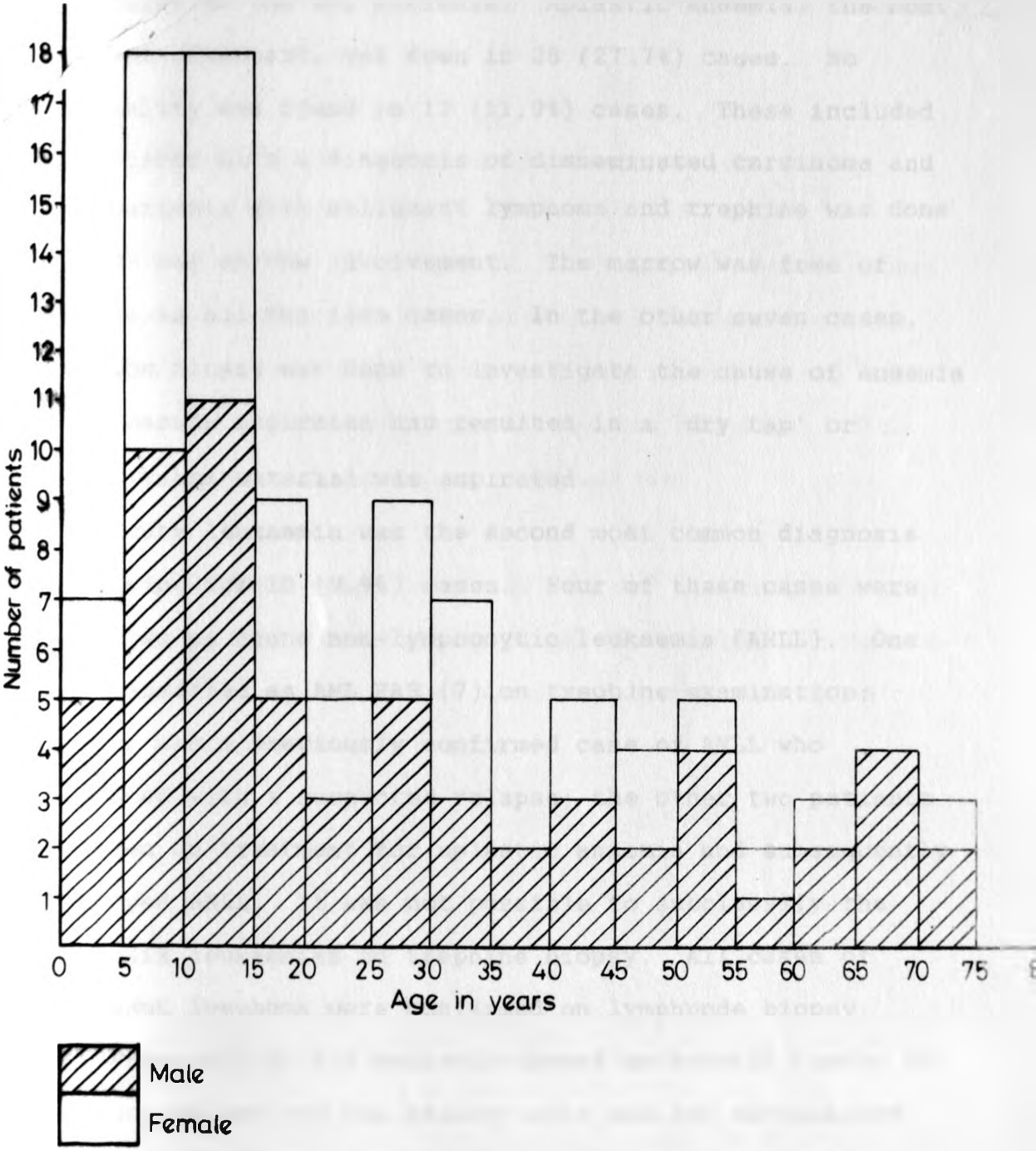


Table 1 is a summary of the frequency of the final diagnoses and the biostatistic data of the different disease categories in the 101 patients. Aplastic anaemia, the most frequent diagnosis, was seen in 28 (27.7%) cases. No abnormality was found in 12 (11.9%) cases. These included one patient with a diagnosis of disseminated carcinoma and four patients with malignant lymphoma and trephine was done to rule out marrow involvement. The marrow was free of disease in all the five cases. In the other seven cases, trephine biopsy was done to investigate the cause of anaemia after marrow aspirates had resulted in a `dry tap' or insufficient material was aspirated.

Acute leukaemia was the second most common diagnosis accounting for 10 (9.9%) cases. Four of these cases were specified as Acute non-lymphocytic leukaemia (ANLL). One was classified as AML FAB (7) on trephine examination; another was a previously confirmed case of ANLL who presented with a suspected relapse; the other two patients had been on treatment for aplastic anaemia and subsequently developed ANLL. It was not possible to subclassify the other six leukaemias on trephine biopsy. All cases of malignant lymphoma were confirmed on lymphnode biopsy.

Three out of six patients showed metastatic tumour in the bone marrow but the primary site was not established. The patient diagnosed to have neuroblastoma on trephine biopsy was later confirmed by skin nodule biopsy. One patient with post-nasal space carcinoma was on treatment with radiotherapy when he developed signs and symptoms of

metastasis. The patient speculated to have prostatic carcinoma had an enlarged prostate per rectal examination, back pain and showed osteosclerotic changes in the lumbar sacral spine on X-ray.

Two patients showed granulomata formation in the marrow, one was confirmed to have acid fast bacilli on sputum smear. The histology of the trephine biopsy showed well formed granulomata with giant cell formation of the Langerhan's type. Ziehl Neelsen stain showed scanty acid fast bacilli. Culture of the marrow was not done. This patient was also found to be HIV1positive.

One patient under investigation for a storage disorder showed gelatinous transformation of the marrow. Niemann's-Picks disease was suspected but liver biopsy and splenic aspirate were however negative.

Table 1: A summary of the frequency of the diagnoses and biostatistic data of the different disease categories in the 101 patients.

DIAGNOSIS ON TREPHINE BIOPSY	AGE IN YEARS		SEX		PATIENTS	
	MEAN	RANGE	M	F	TOTAL	%
APLASTIC ANAEMIA	15.9	2-52	17	11	28	27.7
ACUTE LEUKAEMIA	14.8	2.5-40	9	1	10	9.9
NORMAL BIOPSY	35.5	4.5-58	7	5	12	11.9
NON-HODGKIN'S LYMPHOMA	39.6	4-70	4	1	5	4.9
METASTATIC TUMOUR						
a. POSTNASAL SPACE CARCINOMA	one pt. aged 25		1	0	1	1.0
b. ? PROSTATIC CARCINOMA	" " " 65		1	0	1	1.0
c. SARCOMA-PRIMARY UNKNOWN	" " " 35		0	1	1	1.0
d. NEUROBLASTOMA	" " " 9		0	1	1	1.0
e. CARCINOMA PRIMARY UNKNOWN	70.0	65-75	1	1	2	2.0
MYELOFIBROSIS	63.8	60-75	4	1	5	4.9
REACTIVE MARROW	27.0	4-40	4	1	5	4.9
MYELOPROLIFERATIVE DISORDER	43.3	12-73	2	1	3	3.0
MEGALOBLASTIC ANAEMIA	33.0	25-48	3	0	3	3.0
MYELODYSPLASTIC SYNDROMES	36.7	21-48	2	1	3	3.0
HODGKIN'S LYMPHOMA	12.0	12	1	1	2	2.0
CHRONIC GRANULOCYTIC						

Table 1:(continued)

LEUKAEMIA	15		12-18
GRANULOMA FORMATION (one case confirmed as TUBERCULOSIS)	29		28-30
HAEMOLYTIC PROCESS	8.5		6-11
CHRONIC LYMPHOCYTIC LEUKAEMIA	one pt.	aged	42
PURE RED CELL APLASIA	"	"	7
MULTIPLE MYELOMA	"	"	37
GELATINOUS TRANSFORMATION	"	"	6
DYSPLASTIC CHANGES	"	"	15
IDIOPATHIC THROMBOCYTOPAENIC PURPURA	"	"	29
LYMPHOPROLIFERATIVE DISORDER	"	"	7
MATERIAL INADEQUATE FOR DIAGNOSIS			
TOTAL			

1	1	2	2.0
1	1	2	2.0
2	0	2	2.0
1	0	1	1.0
0	1	1	1.0
1	0	1	1.0
1	0	1	1.0
1	0	1	1.0
1	0	1	1.0
1	0	1	1.0
2	5	7	6.9
62	39	101	100.0

Table 2 shows the quality of the cores obtained in the trephine biopsies.

Seven biopsy specimens were graded as poor in the prospective study. One specimen was misplaced during decalcification process; two were washed away during the staining procedure with scanty invaluable material being left on the slide; four biopsies were reported as showing only bone spicules with no enclosed marrow.

In the retrospective study four biopsies were commented on as being poor cores badly traumatised or processed but an assessment of the residual material was enough to reach a diagnosis.

Table 2: Quality of the trephine biopsy cores.

QUALITY	RETROSPECTIVE	PROSPECTIVE	TOTAL
GOOD	24	37	61
FAIR	11	7	18
POOR	4	7	11
NO COMMENT	11	-	11
TOTAL	50	51	101

Table 3a and b show the different variables commented upon in the trephine biopsy report in the retrospective study.

Only four variables are included here out of the full format used in the reporting of the biopsies (See Materials and Methods).

The quality of the trephine biopsy specimen was the most popular variable commented upon. In only 15 (30%) cases was there a comment on the iron stores.

Of the 50 cases studied, in only 10 (20%) was there a comment on all the four variables.

Table 3a: Frequency of comment items in the trephine biopsy reports in the 50 cases in the retrospective study.

COMMENT ITEM	FREQUENCY	%
QUALITY	39	78
HAEMOPOIETIC ACTIVITY	37	74
RETICULIN PATTERN	25	50
IRON STORES	15	30

Table 3b: Number of comment items recorded in the 50 cases in the retrospective study.

NUMBER OF COMMENT ITEMS	FREQUENCY	%
1	12	24
2	19	38
3	9	18
4	10	20
TOTAL	50	100

Tables 4a-d outline the various indications for the trephine biopsy and the eventual diagnoses. The indications were categorised according to findings of the bone marrow aspirate that necessitated the trephine biopsy to be performed.

a. A `dry tap': This was when no marrow entered the syringe on suction after several attempts at aspiration.

b. Insufficient material: when only fat or blood entered the syringe with or without a paucity of haemotopoietic and/or non-haemotopoietic cells, but the ultimate smear was inadequate for analysis in that a specific diagnosis could not be made.

c. Aspirate suggestive of a specific diagnosis but bone marrow biopsy was required as a complimentary procedure to confirm the diagnosis.

d. An additional category in the prospective study of twelve cases where bone marrow aspirate and trephine biopsy were performed at the same time using the same needle.

A `dry tap' was the commonest indication for needle biopsy - 37 (36.6%) cases. Abnormality was found in 82 (81%) cases, aplastic anaemia being the commonest pathology - 28 (27.7%) cases. In fourteen of these cases the aspirate

was suggestive of the diagnosis but bone marrow was required to confirm the diagnosis. Table 4d shows that ten of the cases were malignant lymphomas (both Hodgkin's and non-Hodgkin's) where trephine biopsy was performed for staging purposes. Trephine biopsy confirmed the marrow aspirate findings in six cases. In four cases the findings differed. The other two cases were diagnostic problems. One had supraclavicular lymphadenopathy biopsy which revealed metastatic squamoid carcinoma. The patient was later found to have carcinoma of the stomach on endoscopy. The other patient was discharged from the hospital for follow-up and a liver biopsy and splenic aspirate planned for a later date.

Table 4a: `Dry Tap' on aspirate as an indication for trephine biopsy and the outcome diagnoses.

DIAGNOSIS ON TREPHINE	RETROSPECTIVE	PROSPECTIVE	TOTAL
APLASTIC ANAEMIA	5	2	7
METASTATIC TUMOUR	4	1	5
MYELODYSPLASTIC SYNDROME	1	1	2
GRANULOMATA FORMATION	1	1	2
NORMAL	2	1	3
MYELOFIBROSIS	3	2	5
ACUTE LEUKAEMIA	4	-	4
LYMPHOMA	1	-	1
MEGALOBLASTIC ANAEMIA	2	-	2
REACTIVE WITH FIBROSIS	1	-	1
MYELOPROLIFERATIVE DISORDER	1	-	1
PURE RED CELL APLASIA	-	1	1
CHRONIC LYMPHOCYTIC LEUKAEMIA	-	1	1
GELATINOUS TRANSFORMATION	-	1	1
TOTAL	26	11	37

Table 4b: Insufficient material on aspirate as an indication for trephine biopsy and the outcome diagnosis.

DIAGNOSIS ON TREPHINE	RETROSPECTIVE	PROSPECTIVE	TOTAL
APLASTIC ANAEMIA	4	3	7
REACTIVE	1	1	2
CHRONIC GRANULOCYTIC LEUKAEMIA	1	1	2
HAEMOLYTIC PROCESS	1	-	1
ACUTE LEUKAEMIA	-	4	4
METASTATIC TUMOUR	-	1	1
LYMPHOMA	-	1	1
NORMAL	3	-	3
TOTAL	10	11	21

Table 4c: Aspirate suggestive of diagnosis but Trephine recommended to confirm diagnosis as indication for Trephine biopsy.

DIAGNOSIS ON TREPHINE	RETROSPECTIVE	PROSPECTIVE	TOTAL
APLASTIC ANAEMIA	9	5	14
LYMPHOMA	1	2	3
MYELODYPLASTIC SYNDROME	1	-	1
HAEMOLYTIC PROCESS	1	-	1
LYMPHOPROLIFERATIVE DISORDER	1	-	1
MULTIPLE MYELOMA	-	1	1
IDIOPATHIC THROMBOCYTOPAENIC PURPURA	-	1	1
NORMAL	1	1	2
TOTAL	14	10	24

Table 4d: Aspirate and biopsy performed at the same time in 12 cases of prospective study.

CLINICAL DIAGNOSIS		DIAGNOSIS ON MARROW ASPIRATE	DIAGNOSIS ON TREPHINE BIOPSY
NON-HODGKIN'S LYMPHOMA	1	*	Y
	2	Y	Y
	3[1]	DYSPLASTIC CHANGES	DYSPLASTIC CHANGES
	4	Y	Y
	5	Y	Y
HODGKIN'S LYMPHOMA	1	Y	Y
	2	*	*
	3	Y	*
	4	Y	*
	5	Y	*
METASTATIC CARCINOMA		*	*
HEPATOSPENOMEGALY? KALA-AZAR? LYMPHOMA		REACTIVE	REACTIVE

Y - INFILTRATE SEEN

* - NO INFILTRATE SEEN

[1] - PATIENT HAD RECEIVED CHEMOTHERAPY BEFORE DIAGNOSIS WAS CONFIRMED.

DISCUSSION

Trephine bone marrow biopsy can be performed in a wide variety of disease conditions as evidenced in this study. In this hospital the major indications are usually a poor yield of the aspirate or a 'dry tap'. Occassionally the aspirate findings may require the trephine biopsy to confirm the pathology.

Out of 101 biopsies studied, the procedure was successful in providing diagnoses in 94 patients. The chances of obtaining a good core on trephine biopsy are high even in the not so experienced hands. Eighty six percent of the cores in the prospective study were either good or satisfactory despite the investigator having performed less than five trephine needle biopsies prior to commencement of the study. Good or optimal cores or specimens are approximately 1.5 - 3.5 cm in length and have a wet weight of about 150 mg (8). After processing, the core should remain intact, at microscopy showing a core of bone spicules with bone marrow enclosed within. The core should of course give sufficient clues for a specific diagnosis to be made. Inadequate specimens obtained in the prospective study resulted mainly due to processing mishaps. Under-sedation and restlessness in patients, especially children may result in inadequate specimens due to poor positioning. The core obtained should be gently teased out of the needle to avoid fragmentation of the specimen.

According to records in the haematology department, KNH, 647 bone marrow aspirates were performed during the seven month period the prospective study was carried out. This gives an overall incidence of 3.4% of `dry tap'. This compares with studies carried out by Mukiibi and others (51) who reported a `dry tap' incidence of 4% among Zimbabweans. Another study (52) showed an incidence of 6.6% but this was mainly confined to aspirates done in lymphomas and carcinomas.

Aplastic anaemia was the most common underlying pathological disorder seen in the trephine biopsies (28%). This also agrees with the Mukiibi study (51) who also found aplastic anaemia to be responsible for the `dry tap' aspiration in 48% of their cases. However, studies from Nigeria (53) found myelofibrosis to be the commonest diagnosis in patients with `dry tap' while Weisberger in USA (54) reported metastatic carcinoma and lymphoma as the most common pathology. This was supported by Engeset et al. in Norway (52).

The myriad of diagnoses on trephine biopsies as exemplified in this study show that the finding of `dry tap' or `scanty yield' on marrow aspiration should not be dismissed as being due to faulty technique or aplasia of the marrow. Because of the patchy involvement of marrow, a normal aspiration may be obtained even if the marrow is involved in certain areas by carcinoma or granuloma. Thus in view of high clinical evidence, a single `dry tap' may be significant of marrow involvement even if subsequent aspirations in the other sites result in normal marrow.

Acute leukaemia may result in `dry tap' or scanty yield (10 (10%) cases in this study) on aspirate. This may be attributed to increased cellular density of the marrow and to the cohesiveness of immature cells (55). This is also true in megaloblastic anaemia (3 (3%) cases).

Bone marrow trephine biopsy is indicated in the staging of malignant lymphomas. In this study trephine biopsy was done in ten cases primarily as a staging procedure. Aspiration was also done at the same time. In 4 cases the trephine biopsy findings differed with the aspirate findings (56). In a study reviewing the non-Hodgkin's lymphomas at KNH 25% of 20 trephine biopsies done in lymphoma patients showed involvement (56). Larger studies involving more patients should be done so as to highlight the usefulness of this procedure in staging of lymphomas.

A review of the format of reporting of the trephine biopsies showed that in only 10 (20%) cases was a full report given to the clinician in the retrospective study. Complete evaluation of the haemopoietic activity, iron stores and reticulin pattern may prove useful to the clinician in the overall management of the patient. It is therefore important that pathologists reporting the biopsies should formulate and adhere to a standard format and emphasize the need to examine all facets of the bone marrow and not just to focus on a striking abnormality thereby missing any associated or incidental findings.

As experienced by the investigator, too often, the technique of trephine needle biopsy had to be explained and demonstrated to clinicians, registrars and nurses in the

wards. One cannot request for an investigation one has no knowledge of. Clinicians need to be made aware of and familiarize themselves with the technique and diagnostic potentialities of the trephine biopsy. The procedure is safe, easy to learn and useful. It assumes even greater importance in our hospitals where expensive and complex diagnostic equipment (like isotope studies, scans, ultrasounds, lymphangiography, etc) is generally lacking and if available, the cost of maintaining it is a major financial constraint to the country's budget.

In Kenya trephine needle biopsy is only confined to a few hospitals. This procedure could easily be introduced and made available to other hospitals in the country especially in the district and provincial hospitals. Now that haematologists and pathologists are being trained to run fully fledged laboratories in the peripheral hospitals, processing and interpretation of the biopsy materials would be easy and fast.

CONCLUSIONS

1. Trephine needle bone marrow biopsy is a relatively simple procedure to perform yielding adequate material for evaluation in most instances.

2. Bone marrow biopsy will most likely provide the diagnosis when bone marrow aspirate fails due to `dry tap' or scanty yield in various conditions.

3. Trephine needle biopsy is indicated in staging of lymphomas.

4. `Dry tap' on marrow aspiration is the commonest indication for trephine biopsy, aplastic anaemia being the commonest underlying pathological disorder.

RECOMMENDATIONS AND FUTURE PERSPECTIVES

This study has attempted to highlight the role and value of trephine needle bone marrow biopsy in various diseases in this hospital. A simple, cost-effective and safe technique, its full potential has yet to be realised. Its use has previously been confined in diagnosis of haematological conditions, the instrument only being available to a few specialists. It is recommended therefore that:

1. Clinicians should familiarize themselves with the technique and its diagnostic potentialities so that more patients may benefit from this procedure.
2. The technique should be made available in other hospitals especially in the provincial and district hospitals.
3. The technique may be adopted as a routine in staging of malignant lymphomas and in diagnosis of suspected cases of aplastic anaemia, the aspirate and biopsy being performed at the same time instead of subjecting the patient to repeated trauma of aspiration then biopsy later.
4. Trephine needle bone marrow biopsies should be reported by both histopathologists in conjunction with haematologists for a more comprehensive outlook.
5. Pathologists and haematologists should formulate and adhere to a standard format for reporting on the trephine biopsies so that full information is given to

clinician who can then correlate the biopsy findings with the clinical findings and reach a final diagnosis.

CONSTRAINTS

The major problem was the availability of the Jamshidi trephine biopsy needle. The department of Human Pathology had only one needle which was old and had been sharpened several times therefore it easily bent when used. The needle had to be sterilized in the Central Sterilizing Unit of the hospital and this would take a minimum of two days. Another needle, therefore had to be purchased abroad and this took some time.

Another problem was the shortage of several items experienced in the ward. Sometimes analgesics were not available immediately especially for paediatric patients who needed sedation with intravenous pethidine and valium. This often necessitated the postponement of the procedure. At times the nurse on duty not knowing what a trephine biopsy entails was not ready at the time the biopsy was to be performed, having several required items missing. The problem was overcome by informing the nurse prior to performing the procedure as to what was required.

Several problems were encountered during the processing of the specimens. One specimen was misplaced during decalcification and another was washed away during the staining process. These biopsies could not be repeated as one patient had been discharged from the hospital and the other had died. Some specimens that appeared good on

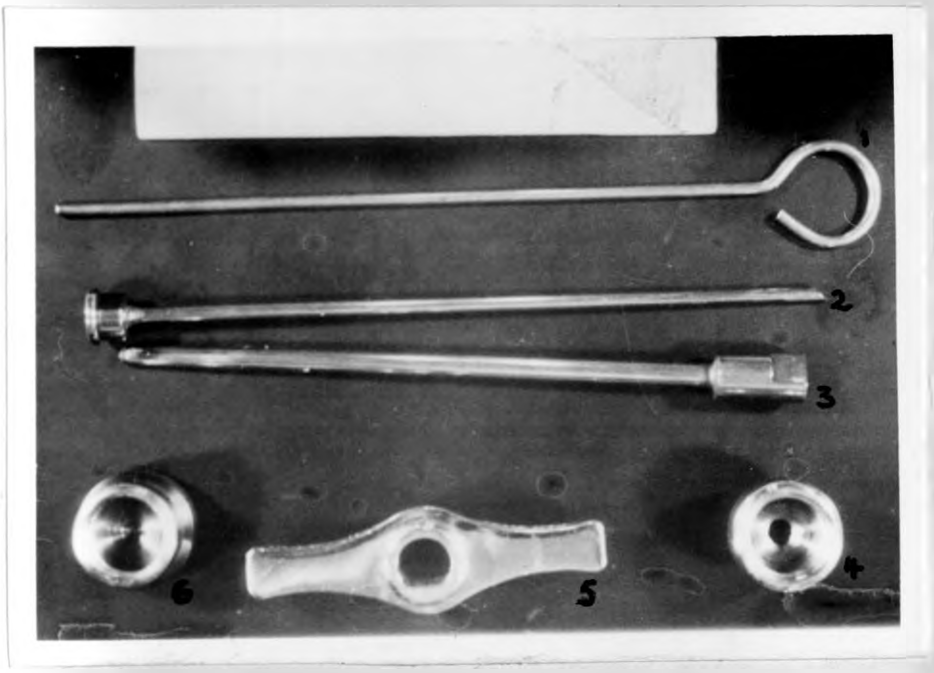
gross examination were mishandled during processing ending up with inadequate material on microscopic examination. Some of the special stains were poorly done and necessitated a repeat.

A major obstacle encountered was in the Records Department. Retrieval of retrospective clinical notes was painstakingly slow due to wrong coding of patient file numbers.

It is therefore suggested that trephine biopsy needles should be made available as part of the diagnostic tools available for use by doctors. The minimum cost per needle is approximately KSh. 6,500.00 (1979 price in United Kingdom). The longevity of the needle has already been described.

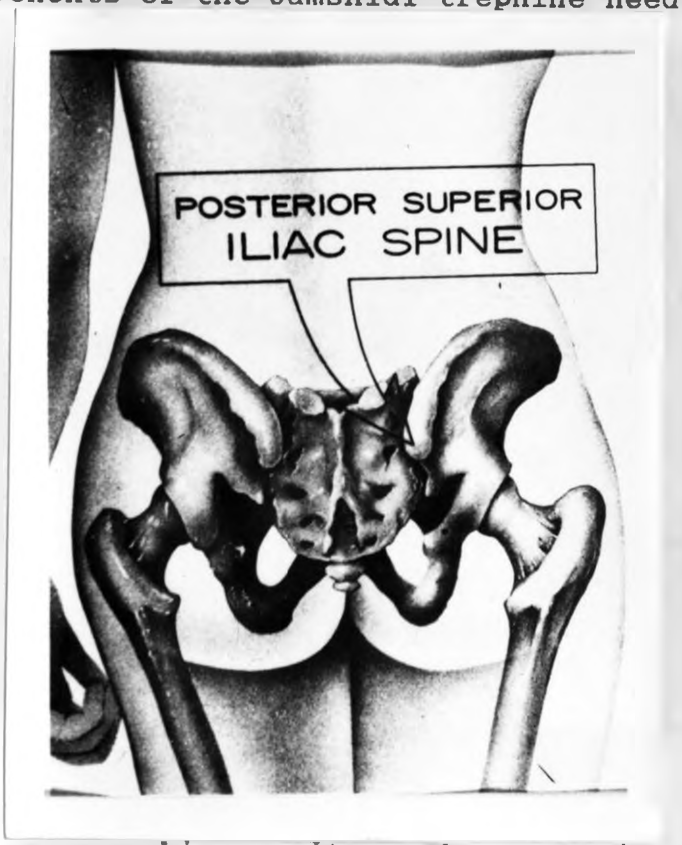
As the biopsy materials are delicate it is suggested that the specimens be processed by an assigned qualified technician and they should not be included among the routine processing procedure to minimize mishandling.

Records are an important part of any establishment. The machinery of the records department should be looked into and overhauled. Computerization may be the answer in the near future.

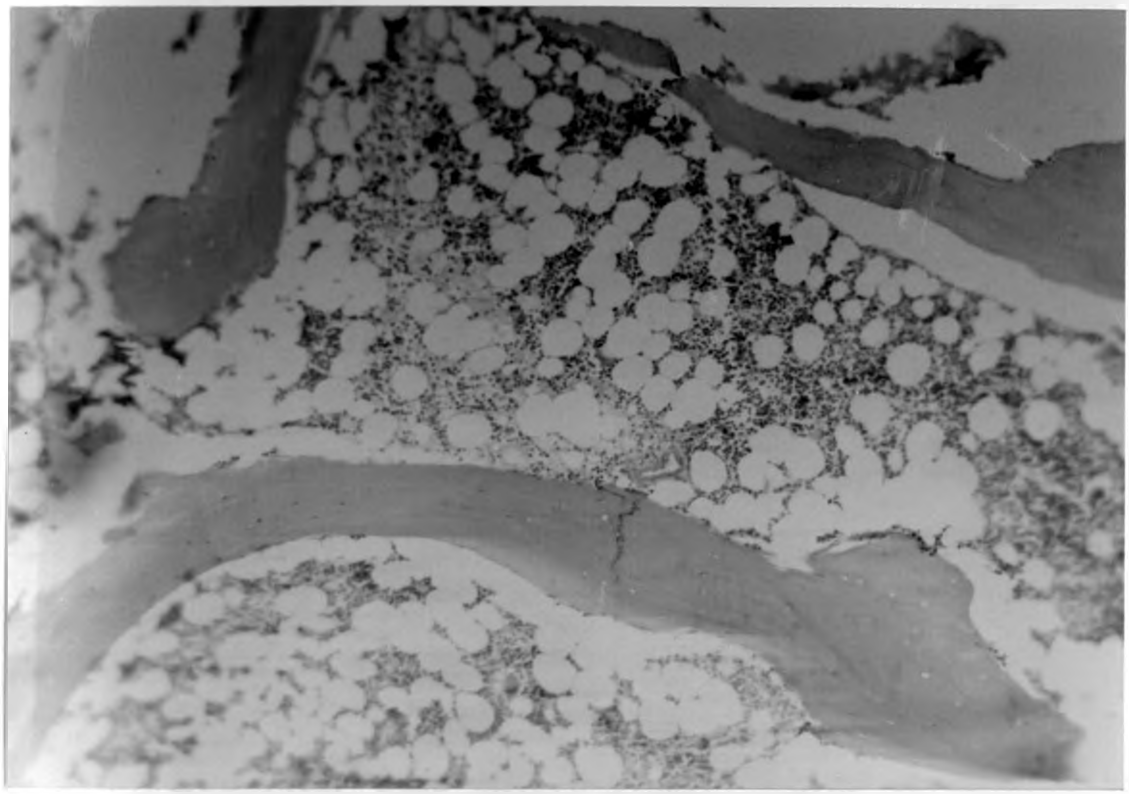


1. Probe
2. Stylet
3. Cannula
4. Cannula retainer
5. Handle
6. Comfort knob

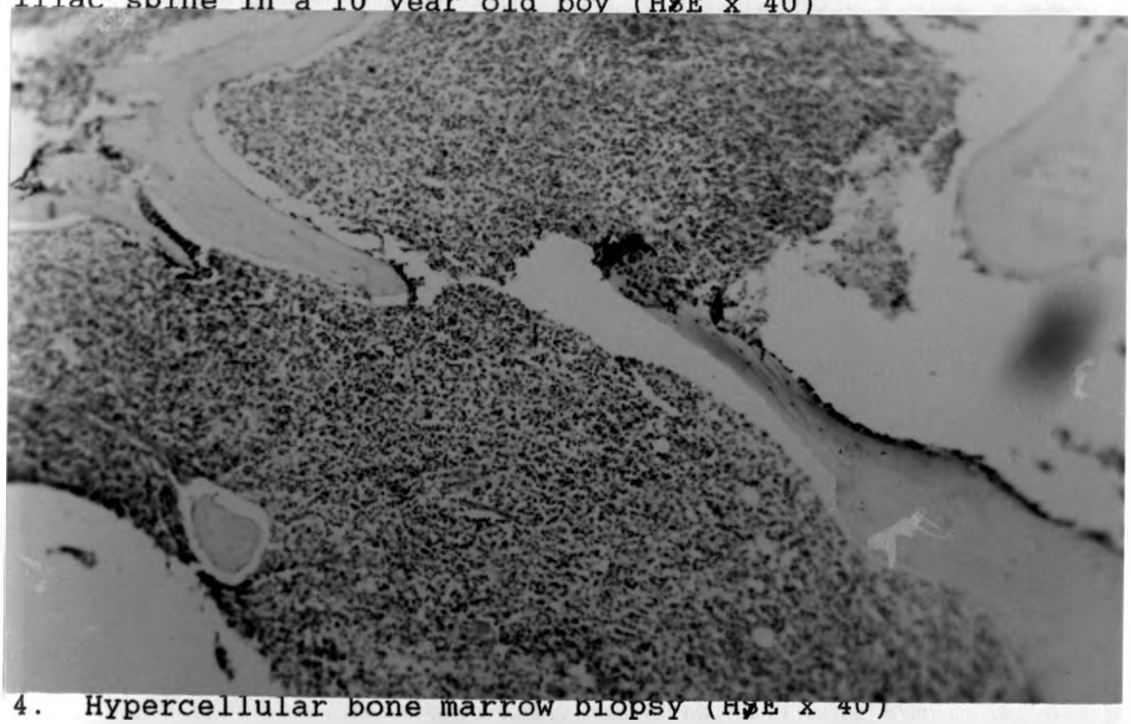
1. Components of the Jamshidi trephine needle



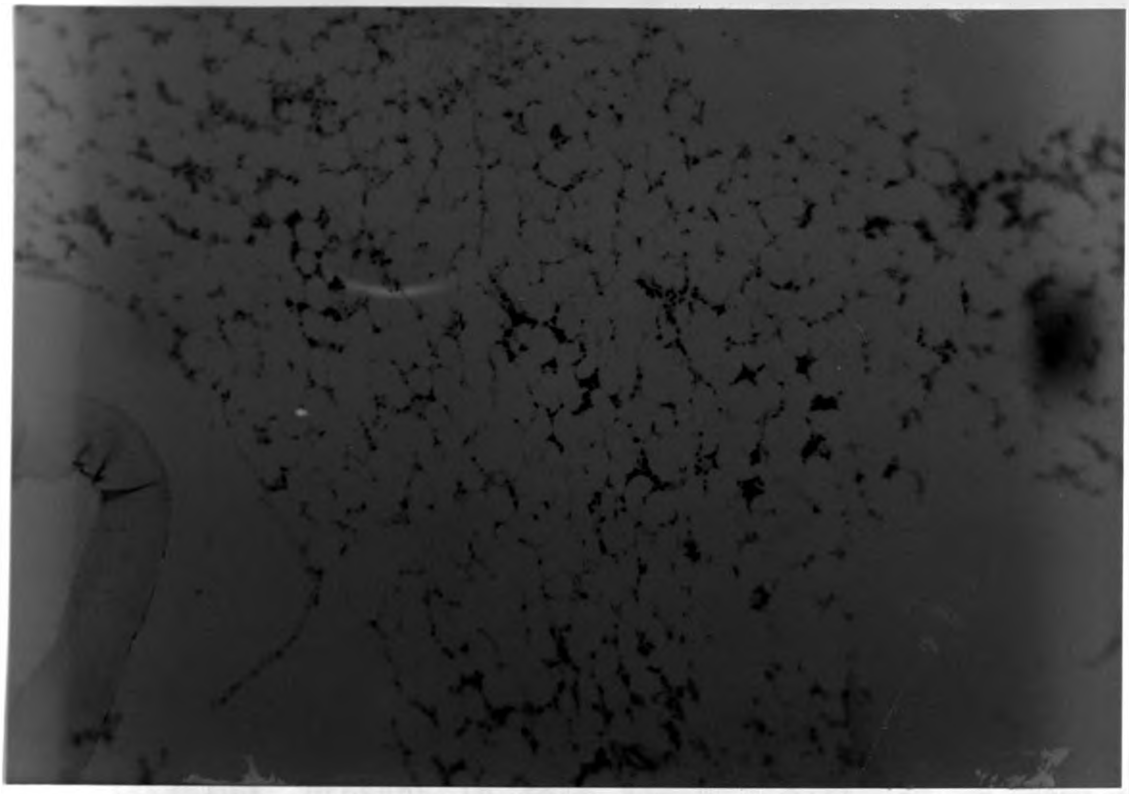
2. Bone marrow biopsy site. The posterior, superior iliac spine is used as the site for both the aspirate smear and core biopsy.



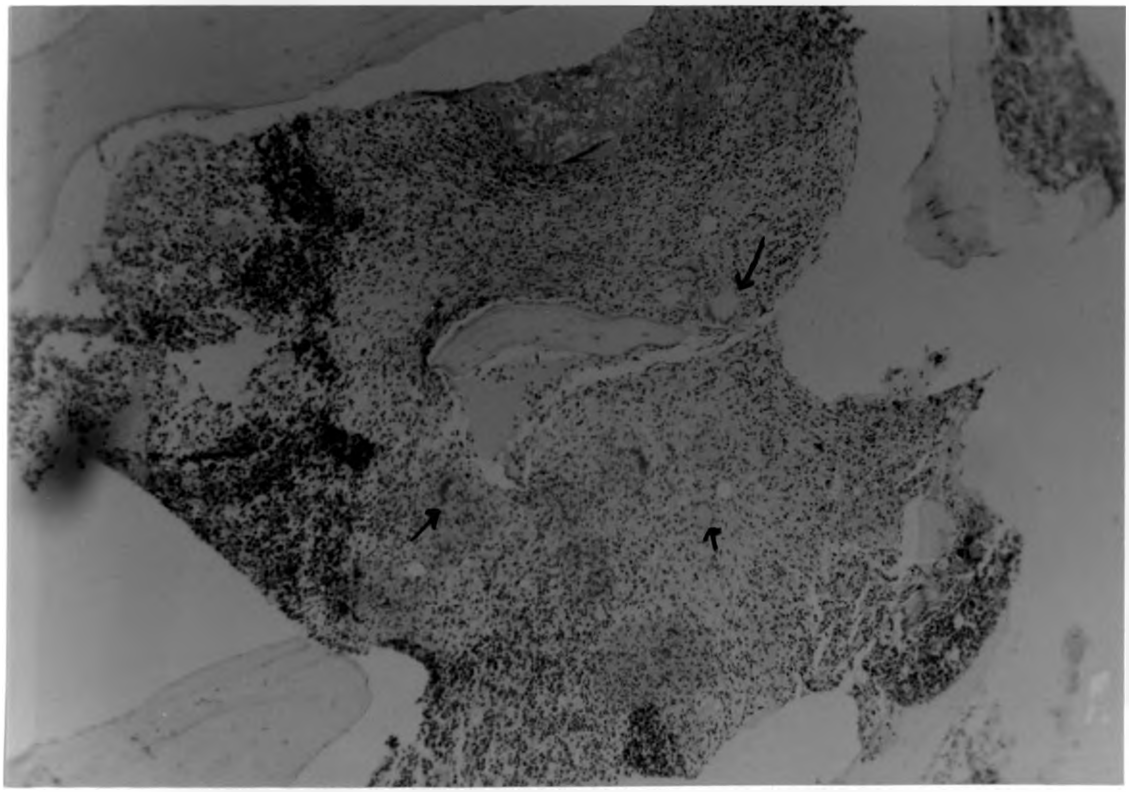
3. Normocellular bone marrow biopsy from the posterior iliac spine in a 10 year old boy (HØE x 40)



4. Hypercellular bone marrow biopsy (HØE x 40)

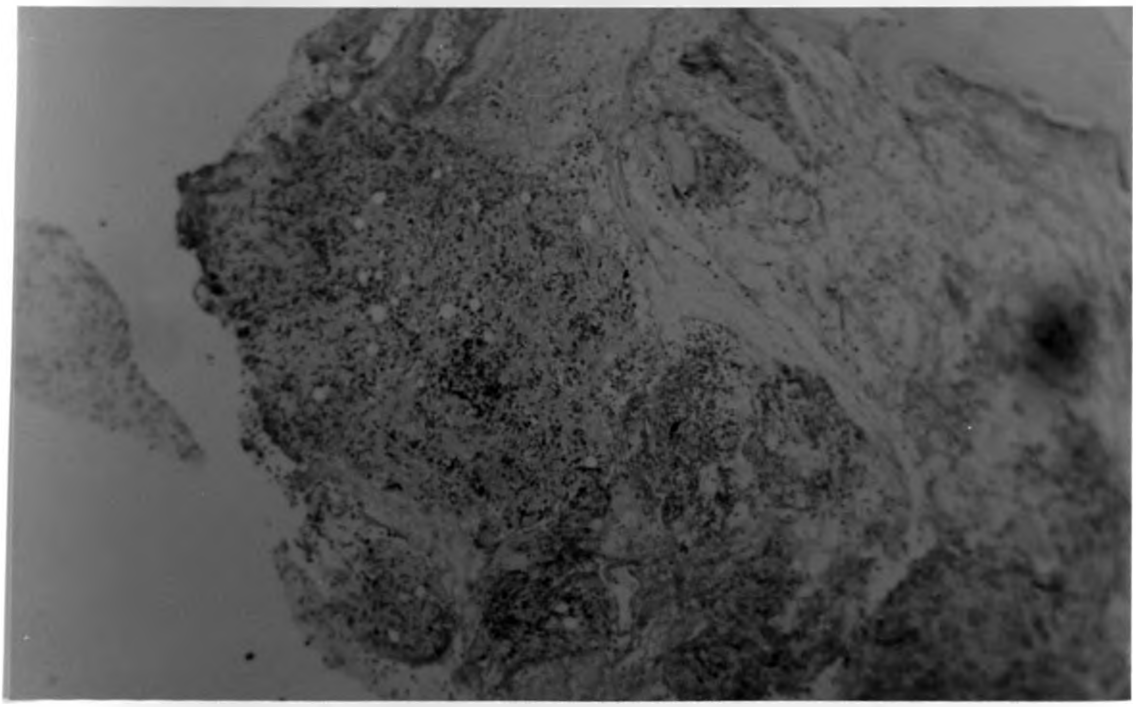


5. Hypercellular bone marrow biopsy. Over 90 percent of this specimen is comprised of fat cells (H&E x 40)

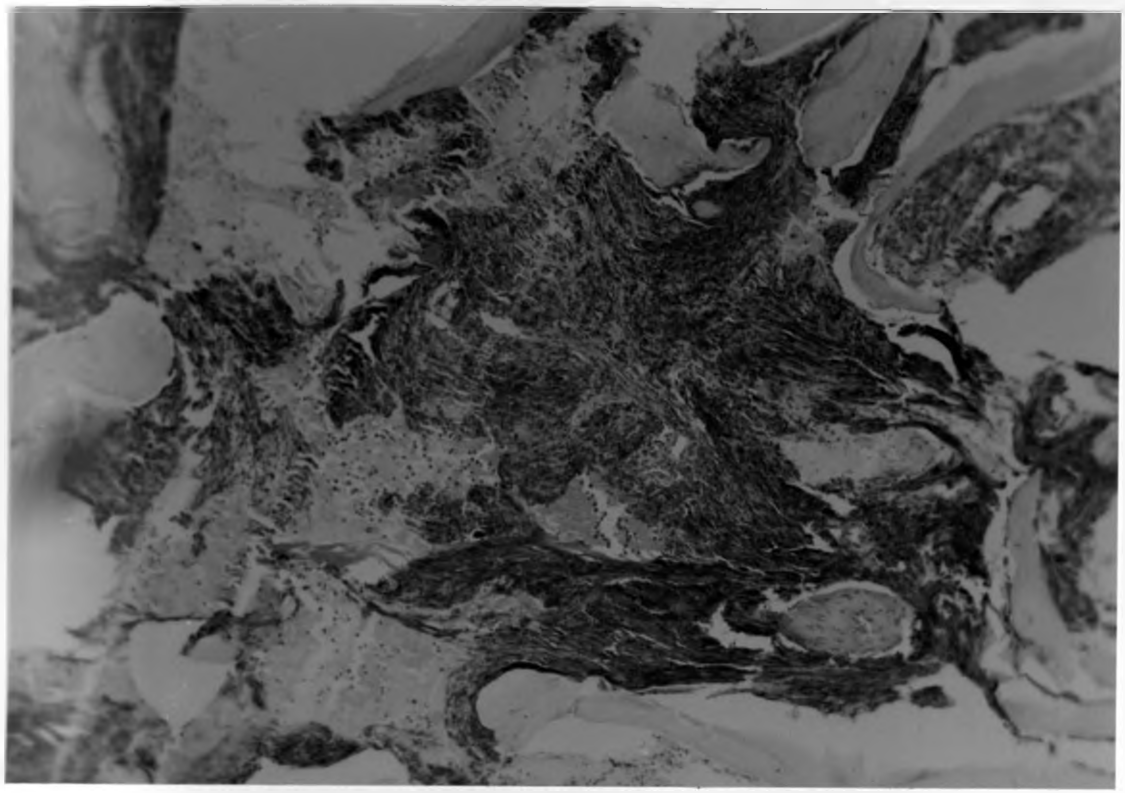


6. Bone marrow granuloma with giant cell formation (arrows). This patient was confirmed to have Tuberculosis (H&E x 40) The section shows folding artefact on the right side.

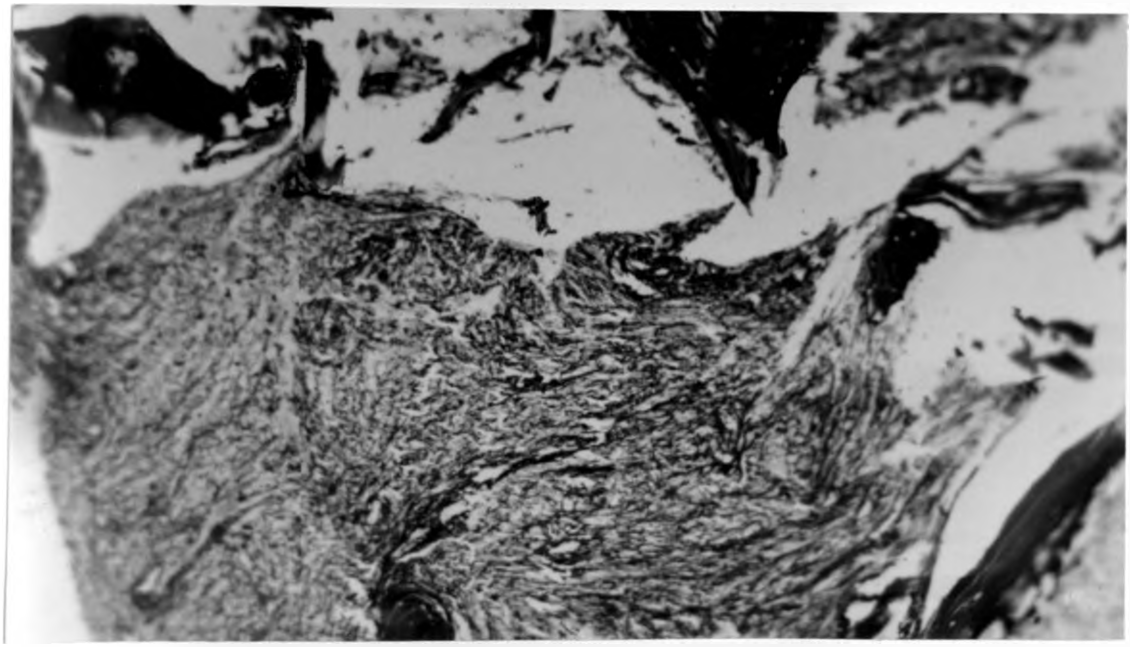
7. Hodgkin's disease. A fibroblastic and cellular infiltration present in this field. Although no obvious Reedsternberg cell is demonstrable, this is consistent with Hodgkin's involvement of the marrow (H/E x 100)



8. Metastatic carcinoma. Solid sheets of tumour cells infiltrating most of the biopsy specimen (H & E x 40)



9a. Myelofibrosis (H/E x 40).



9b. Myelofibrosis. Reticulin stain demonstrates greatly increased numbers and thickness of fibres Gordon Sweet reticulin stain (H/E x 100)

53

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APPENDIX I

STUDY PROFORMA SHEET

NAME:_____AGE_____SEX_____

UNIT NO_____WARD_____

BRIEF CLINICAL HISTORY_____

_____

PROVISIONAL CLINICAL DIAGNOSIS AND CURRENT TREATMENT:

_____

INDICATION FOR BONE MARROW TREPHINE BIOPSY:

_____

_____

PERIPHERAL BLOOD FILM (pbf) AND BONE MARROW EXAMINATION

FINDINGS:_____

_____

PBF

Hb:_____g/dl. RBC: _____x 10^9/dl

RBC:_____x 10^9/dl. Platelets:_____x 10^9/dl.

Differential WBC:_____

Film: _____

Bone Marrow Aspirate and Bone Marrow Trephine:

Quality_____

Cellularity_____Myeloid/Erythroid ratio.

Erythropoiesis:_____

Leucopiesis:_____

Megakaryocytes:_____

Plasma Cells:_____

Abnormal Cells:_____

Iron:_____

Plus on trephine:_____

Stromal Abnormalities:_____

Vessel Abnormalities, bone marrow changes:_____

_____

_____

APPENDIX II

May Grunwald Giemsa's Stain

1. Prepare blood films on clean, dust and grease-free slides.
2. Air dry the films.
3. Fix film by immersing in jar of methanol for 10 - 20 minutes.
4. Put slide into staining jar containing May-Grunwald's stain freshly diluted with an equal volume of buffered water - 15 minutes.
5. Transfer slide, without washing into jar containing Giemsa's stain freshly diluted with 9 volumes of buffered water - 10 - 15 minutes.
6. Wash rapidly in 3 or 4 changes of water and finaly allow to stand undisturbed in water for differentiation to take place (2 - 5 minutes).
7. Stand slide upright to dry.
8. When thoroughly dry, the slides may be mounted with a cover glass using a mountant.

APPENDIX III

Technique for Biopsy and Aspiration Using the Jamshidi Needle.

1. The patient is placed in a right or left lateral decubitus position with his back comfortably flexed and his knees drawn towards his chest.

2. The posterior superior iliac spine is located by palpation and marked.

3. With the use of a sterile technique, the skin is prepared with antiseptics and draped.

4. The skin over the marked area, as well as the deeper tissues and especially the periosteum, are infiltrated with local anaesthetic.

5. A 3 mm skin incision is made with a scapel blade.

6. The biopsy needle with the stylet locked in place is advanced through the incision, pointing toward the anterior superior iliac spine, into the bone cortex.

   Entrance into the marrow cavity is generally detected as a decreased resistance.

7. Marrow can then be aspirated at this point. The stylet is removed and a 10 - 20 ml syringe is attached and 1 -

2 ml of marrow are aspirated. Aspirated particles are expelled from the syringe onto alcohol cleansed microscope slides. These are quickly tilted and excess blood removed using a sunction bulb, small capillary tube or 2 ml syringe. This leaves pale, grey-white marrow fragments and a small amount of blood.

A second microscope slide is then placed on top of the first and a smear is made by pulling the slides apart in a sliding motion.

8. The biopsy needle is slowly and gently advanced with smooth clockwise - counter clockwise motions until adequate marrow is obtained.

9. The biopsy needle is then slowly removed with alternating rotary motions.

10. The specimen obtained is gently removed with a long probe which must be introduced through the distal end. The biopsy specimen can be expelled easily through the proximal end. The specimen is used for making trails where necessary on alcohol - cleansed microscope slides and then fixed in.

Ideal specimen size should be 1.5-3.5 cm long. The trails are stained by MGG stain. Haematoxylin Eosin (H/E) and iron stains are prepared on each section.

11. Post-biopsy Care of Patient

This ordinarily consists of applying pressure over the posterior illium for about 60 minutes, which is accomplished with a pressure dressing, and having the patient lie incumbent in bed. Patients with bleeding tendencies or other complications are carefully observed for longer periods. Analgesics are seldom necessary following the biopsy procedure.

Note: Paediatric patients upto 13 years old are sedated by intravenous diazepam 0.5 mg/kg and pethidine 2 - 5 mg/kg prior to performing the procedure.

If necessary some adult patients may require sedation.

APPENDIX IV

Preparation of the bone marrow biopsy

Immediately following the bone marrow biopsy, fix the specimen in 10% formol saline for 24 hours. It is decalcified using 10% formic acid for 24 hours and is then rinsed in running tap water for 2 hours. It is processed in the usual manner through graded alcohols and acetone. After embedding the tissue in paraffin, sections are cut at 5 to 6 um.

Haematoxylin and Eosin Method

1. Bring section to water.
2. Stain in Mayer's haematoxylin for 10 minutes.
3. Wash in water
4. Differentiate in 1% acid alcohol
5. Wash in water
6. Blue in Scott's tap water for 30 minutes
7. Rinse in water
8. Counterstain in 1% eosin for 5 minutes
9. Rinse in water
10. Dehydrate in alcohol
11. Clear in xylene
12. Mount in Dibutylphthalate Xylene (DPX).

Perls Prussian Blue Reaction (For Iron Stain).

Solutions

1. 4% aqueous potassium ferrocyamide (Solution I)
2. 4% aqueous hydrochloric acid (Solution II).
3. 0.5% neutral red.

Technique

1. Bring sections to water.
2. Rinse with distilled water.
3. Flood sections with a mixture of equal parts of solutions I and II freshly prepared - 15 minutes.
4. Rinse in distilled water.
5. Counterstain with 0.5% neutral red for 30 seconds.
6. Rinse in distilled water.
7. Dehydrate in 3 changes of absolute ethanol.
8. Clear in 3 changes of xylene.
9. Mount in Dibutylphthalate Xylene (DPX).

Gordon and Sweet's Method

Solution A: Acidified Potassium Permanganate solution 1% potassium permanganate - 47.5 ul 3% sulphuric acid - 2.5 ml.

Solution B: 1% aqueous oxalic acid.

Solution C: 2% Ferric Ammonium Sulphate (Iron Alum)

Staining Technique:

1. Bring sections to distilled water
2. Oxidise in Sol. A for 2 minutes wash in water.
3. Bleach in 1% oxalic acid until sections all clear.
4. Wash in tap water and rinse in distilled.
5. Sensitize in 2% iron alum for 10 minutes.
6. Rinse in distilled water.
7. Impregnate in ammoniacal silver solution for 30 seconds.
8. Wash in distilled water and reduce formalin.
9. Wash in distilled water and tone in gold chloride for 1 minute.
10. Wash in water and fix in hypo for 5 minutes.
11. Wash in running tap water for 5 minutes.
12. Counterstain in neutral red for 30 seconds.
13. Wash in water.
14. Dehydrate in 3 changes of ethyl alcohol.
15. Clear in 3 changes of Xylene.
16. Mount in Dibytylphthalate Xylene (DPX).

Periodic Acid Schiff Method (PAS):

Staining Technique:

1. Bring sections to water.
2. Oxidise for 10 minutes in 1% aqueous periodic acid.
3. Wash in running tap water for 5 minutes.
4. Rinse in distilled water.

5. Place in Schiff's reagent for 15 minutes or until the sections turn magenta in colour.
6. Rinse in 3 changes of freshly prepared 0.5% Sodium metabisulphite.
7. Wash in running tap water for 10 minutes.
8. Stain the nuclei in alum haematoxylin for 7 minutes.
9. Differentiate in 1% acid alcohol - 3 dips.
10. Blue in running tap water for 10 minutes or Scott's tap water for 1 minute.
11. Counterstain in 0.3% tartrazine in cellosolve for 3 minutes.
12. Dehydrate in absolute ethanol, clear in Xylene and mount in DPX.

Masson Trichrome

Staining Technique

1. Bring sections to water
2. Stain the nuclei with celestin blue - haematoxylin sequence, that is celestin blue for 5 minutes and Mayer's haematoxylin for 5 minutes.
3. Differentiate in 1% acid alcohol until only the nuclei are stained.
4. Blue in running tap water for 10 minutes.
5. Stain in 1% Ponceau 2R for 5 minutes.
6. Rinse in distilled water.
7. Mordant in phosphomolybdic acid for 5 minutes.
8. Rinse in distilled water.
9. Counterstain in 2% light green for 3 minutes.

10. Rinse off excess stain in distilled water.
11. Wash well in 1% acetic acid to remove excess light green from the cytoplasmic structures.
12. Dehydrate in 3 changes of absolute ethanol.
13. Clear in 3 changes of Xylene.
14. Mount in DPX.

APPENDIX V

An example of a complete report of the trephine biopsy is included.

A brief account of the case is given.

A 61 year old female with 5 months history of general malaise, palpitations, abdominal pain and swelling. Physical examination revealed moderate wasting, moderate pallor and discrete axillary and inguinal lymphnodes. Gross hepatosplenomegaly.

A provisional clinical diagnosis of anaemia and hepatosplenomegaly 2^{o} to a myeloproliferative disorder? Chronic myeloid leukaemia was made.

Peripheral Blood Examination

Haemoglobin - 5.5 g/dl

Packed cell volume - 0.43

Mean corpuscular haemoglobin concentration - 29 g/dl

WBC 66 x 10^{9}/l

RBC 1.11 x 10^{12}/l

Platelet Count 40 x 10^{9}/l

Film RBC

Normocytic Normochromic

Numerous normoblasts = 130/100 WBC (Approximately)

No Malaria parasites seen

WBC

Left shift

Polymorphs 50% lymphocytes 32%

Eosinophils 2% Band forms 10%

Metamyelocytes 6%

Comment

Leucoerythroblastic response.

Bone Marrow Aspirate Report

Scanty particles present with very few cellular elements present which show reduced erythropoiesis which is normoblastic.
Myelopoiesis appears increased but difficult to assess.
Hardly any megakaryocytes are seen.
Iron stain is not assessable.

Comment

Inadequate material which is non diagnostic.

Trephine Needle Biopsy Report

Adequate core which shows replacement of marrow by dense fibrous tissue.

Occasional foci of haemopoetic activity are seen scattered within. A few megakaryocytes are seen. This is no increase in plasma cells nor is there any abnormal cellular infiltrate. No parasites are seen. Stainable iron is decreased. Reticulin stain shows a diffuse increase in coarse reticulin fibres. Masson trichrome stain shows mild collagenization.

Comment

Findings are in keeping with myelofibrosis.

APPENDIX VI

List of the minimum requirements for establishing the trephine needle biopsy procedure:

1. Trephine biopsy needle
2. Sterile incision trays
3. Antiseptics
4. Analgesics - Pethidine and intravenous valium
5. A funtioning histopathology and haematology laboratory
6. Qualified haematologist/Pathologist.

APPENDIX V11

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KENYA

ur Ref:

our Ref:

25/9/89

Dr. J.A. Rajab
Dept. of Pathology

Dear Dr. Rajab,

REF: THE VALUE OF TREPHINE NEEDLE BONE MARROW BIOPSY IN THE EVALUATION OF VARIOUS HAEMATOLOGICAL AND NON-HAEMATOLOGICAL CONDITIONS AT K.N.H, KENYA

This is in response to your request for clearance. I am glad to inform you that you have been cleared to embark on the above study.

Yours sincerely,

DR. D.M. NJAI
Ag. Chairman
Ethical & research committee.

cc: Director, K.N.H
Deputy director, K.N.H