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FROZEN BONE MARROW TREPHINE BIOPSY – A TECHNICAL EVALUATION

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Summary

The routine study of bone marrow trephine biopsies involves fixation, decalcification, paraffin-embedment, sectioning and staining. However, this process creates artifacts, produces shrinkage of tissue, consumes time and can result in sections of unsatisfactory cytological quality. It also renders the tissue unsuitable for enzyme-histochemical and immunohistochemical analyses. Frozen section of bone marrow without decalcification was evaluated as an alternative method for the study of bone marrow. This method was found to give sections with comparable cytological quality to that of paraffin-embedment, yielded sections for interpretation within 24 hours, and allowed enzyme-histochemical and irnmunohistochemical analyses to be applied successfully.

Keywords: Frozen section, bone marrow, trephine biopsy, enzyme-histochemistry, immuno-histochemistry.

INTRODUCTION

Interest in the study of bone marrow bloomed in the 1930's when bone marrow was aspirated from the sternum with an adapted lumbar puncture needle. For the past 50 years, bone marrow aspiration cytology has been the basis of morphological investigation and diagnosis of blood disorders. However, even in the best aspirate preparations, the spatial distribution of the haemopoietic cells and the anatomical relationship between cells and stromal elements (fatty, vascular, reticular and bony) can never be observed and assessed accurately. Furthermore, the technique often fails to recognise certain disease states such as Hodgkin's disease, non-Hodgkin's lymphoma, granulomatous diseases, metastatic carcinoma and myelofibrosis. This limitation has led to the increased use of trephine biopsy of the bone marrow and the analysis of bone marrow sections by histological techniques. Nowadays many haematology laboratories advocate both aspiration and trephine biopsies for the routine study of bone marrow and haematologists have also begun to acquire skills in the interpretation of both bone marrow aspirate smears and trephine sections.[1,2,3]

Bone marrow trephine biopsy specimens are routinely fixed, decalcified, paraffin-embedded, sectioned and then stained. In so doing, the method creates artifacts, produces tissue shrinkage and often results in poor cytological quality. In addition, the properties, especially that of enzyme content of the tissues, are modified or destroyed. Over the years, newer techniques have been introduced. The technique of embedding undecalcified iliac crest biopsies in plastic was introduced by Burkhardt in 1956. This method has now been perfected and is capable of producing semithin sections that, after staining, give excellent cytological quality.[4] However, it is expensive, laborious and time-consuming (over four days are required compared to the two days of traditional paraffin-embedding). In addition, the technique damages enzymes and induces loss of specific antigenic determinants. However, it is not impossible to use enzyme and immunohistochemical methods on the bone marrow and, in fact, some success have been reported.[5,6]

Other techniques are continually being explored. An ideal technique has to fulfil the following objectives:

1. It should produce cytological quality better than paraffin-embedded sections and be as good as, if not better than, plastic-embedded sections.
2. It should be rapid, preferably as fast as the aspirate technique i.e. within 1 day of processing time.
3. It should allow enzyme and immuno-histochemical analyses on the sections.

The cyrostat technique nas been shown to be the most suitable for producing tissue sections for enzyme and immunohistochemical analyses.[7] It has recently been applied to the bone marrow.[8,9] We have evaluated this technique in our laboratory from January 1987 to June 1988 and report our findings.

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MATERIALS AND METHODS

A. SAMPLES

13 selected specimens were obtained on routine requests for trephine biopsies. Each biopsy specimen was divided transversely into two halves: one half was processed traditionally using the paraffin-embedded method in the routine histopathology laboratory; the other half was sectioned with a cryostat and processed in the haematology research laboratory. In this way, routine histopathology was not disrupted and its products served as controls.

The specimens came from patients with haematological disorders as listed in Table 1.

TABLE 1
HAEMATOLOGICAL DISORDERS STUDIED

Diagnosis	No. of cases
Aplastic anaemia	1
Myelodysplastic syndrome	1
Polycythaemia rubra vera	1
Myelofibrosis	2
Tumour metastasis	2
Malignant lymphoma	3
Chronic myeloid leukaemia	1
Nutritional anaemia	2

B. TECHNIQUES

1. *Bone Marrow Trephine Biopsy.*

This was carried out using a Jamshidi needle on the posterior iliac crest of the patient under local anesthesia with 2% lignocaine injection. The bone marrow core was cut into two halves transversely using a scalpel. One half was immersed in Bouin's fixative for routine histopathology and the other half kept in 1% gum-sucrose solution at 4°C for 3 to 4 hours to allow the solution to infiltrate the tissue.

2. *Frozen Section with Cryostat.*

If the specimen was not processed immediately, the tissue was stored in a –20°C freezer. It was then cut in a cryostat after blocking in OCT compound. Multiple sections were air-dried on glass slides before immersing into appropriate fixative at 4°C for 5 minutes. For haematoxylin & eosin, immunochemical and reticulin stains, Bouin's fixative was used; for enzyme histochemical analyses, neutral 10% calcium-formalin was used.

Unfixed sections were wrapped in aluminium foil and stored at –20°C until later. When used, the slides were brought to 4°C before being unwrapped and fixed as above.

3. *Staining Methods*

The stains used were as shown in Table 2.

TABLE 2
STAINS USED FOR FROZEN TREPHINE SECTIONS

Stain	Method
Haematoxylin and Eosin	Bancroft and Steven (1982)[10]
Reticulin	Gordon and Sweets (1936)[11]
Dual esterases	Li, Yam and Crosby (1972)[12]
Acid phosphatase	Goldberg and Barka (1962)[13]
Alkaline phosphatase	Rutenberg (1965)[14]
5'-Nucleotidase	Silber et al. (1975)[15]
Leder-Giemsa	Cheong and Chong (1986)[16]
Peroxidase	Kaplow's method (1965)[17]
Immunoalkaline phosphatase*	Sun, Li and Yam (1985)[18]

*Monoclonal antibodies were from Dako.
Antibodies tested were: T cell (CD2), B cell (CD22), common ALL (CD10), LCA (CD45), myeloid cells (CD15) and megakaryocytes (CD w 41).

RESULTS

1. *Frozen Sections*

The cryostat gave excellent sections comparable to those obtained from paraffin embedment. Fragmentation of some sections, however, did occur especially when the biopsy core contained a lot of cortical bones. Tearing of sections most commonly occurred next to the bony trabeculae.

2. *Results of Staining*

The results of histochemistry and immunochemistry stains on frozen and paraffin sections are summarised in Table 3.

DISCUSSION

This study showed that the cryostat technique produced sections with cytological quality comparable with paraffinembedded sections, yielded results within 1 day and allowed the successful application of enzyme-histochemistry and irnmunohistochemistry. The technique is reliable and informative. It is particularly useful in situations where the aspirate technique yielded only blood or was a *punctio sicca* and urgent information is needed for patient management.

The procedure adopted allows the option to use different fixatives at a later stage to suit the requirement of the stains. Besides, the procedure does not require organic solvents

TABLE 3
RESULTS OF HISTOCHEMICAL AND IMMUNOCHEMICAL STAINS ON FROZEN SECTIONS COMPARED WITH PARAFFIN SECTIONS

Stain	Observations
H & E	Comparable with paraffin sections (Figs. 1 & 2). Less shrinkage artifact. Fat cells intact. Cytoplasm better visualised.
Reticulin	Comparable with paraffin sections (Fig. 3)
Chloracetate esterase	Comparable with paraffin sections fixed in calcium-formalin. Did not work on paraffin sections fixed in Bouin's fixative.
Dual esterase	Good results. Did not work on paraffin sections.
Acid phcsphetase	Variable staining, most cells were labile to tartrate. Did not work on paraffin sections.
Alkaline phosphatase	Demonstrated well. Vasculoendothelial cells stained strongly. Granulocytic series were unstained. Useful stain to show boce marrow architecture. Did not work on paraffin sections.
5'-Nucleotidase	Malignant B cells were negative staining when compared to reactive lymphoid aggregates. Did not work on paraffin sections.
Leder-Giemsa	Comparable to paraffin sections fixed in calcium-formalin. Did not work on paraffin sections fixed in Bouin's solution.
Peroxidase	Stained well. Did not work on paraffin sections.
Immunoalkaline phosphatase	Good results (Fig. 4). Monoclonal antibodies used were not meant for paraffin sections.

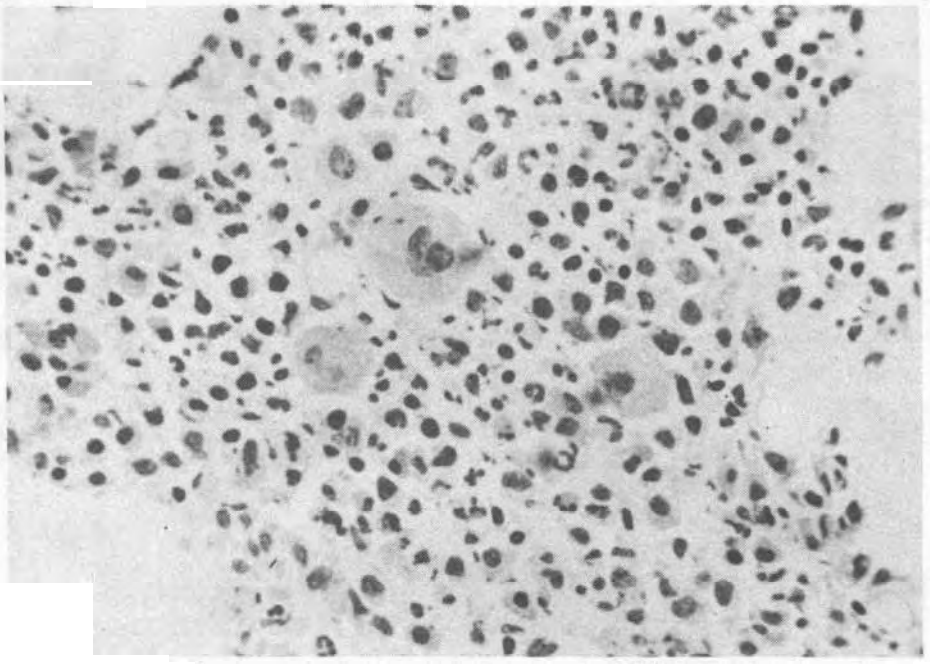

FIG. 1: Frozen section of bone marrow stained with Haematoxylin and Eosin (x 400).

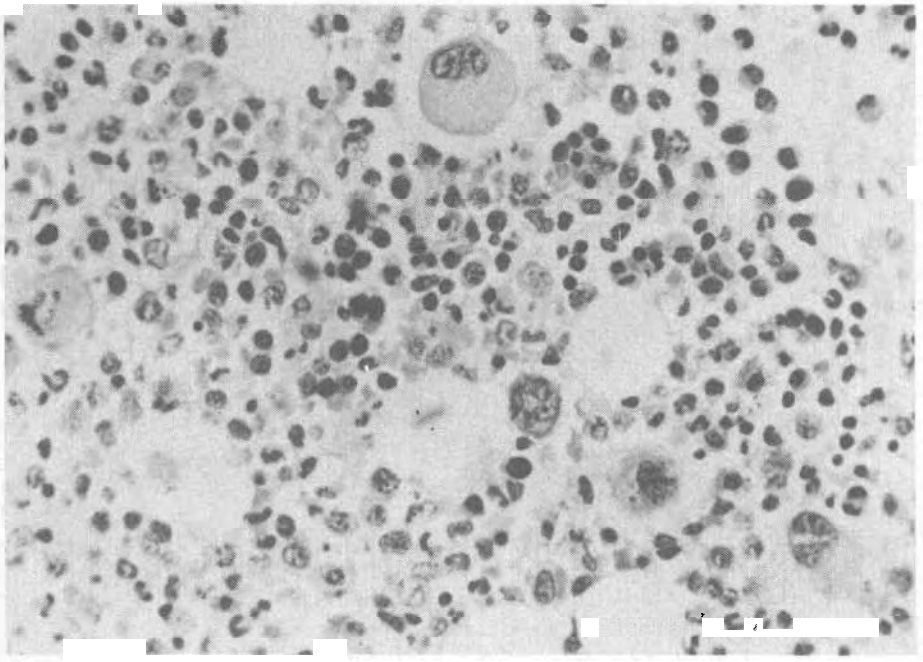

FIG. 2: Paraffin-embedded section of bone marrow stained with Haematoxylin and Eosin (x 400). Note the shrinkage artifact, loss of fat cells and distorted morphology.

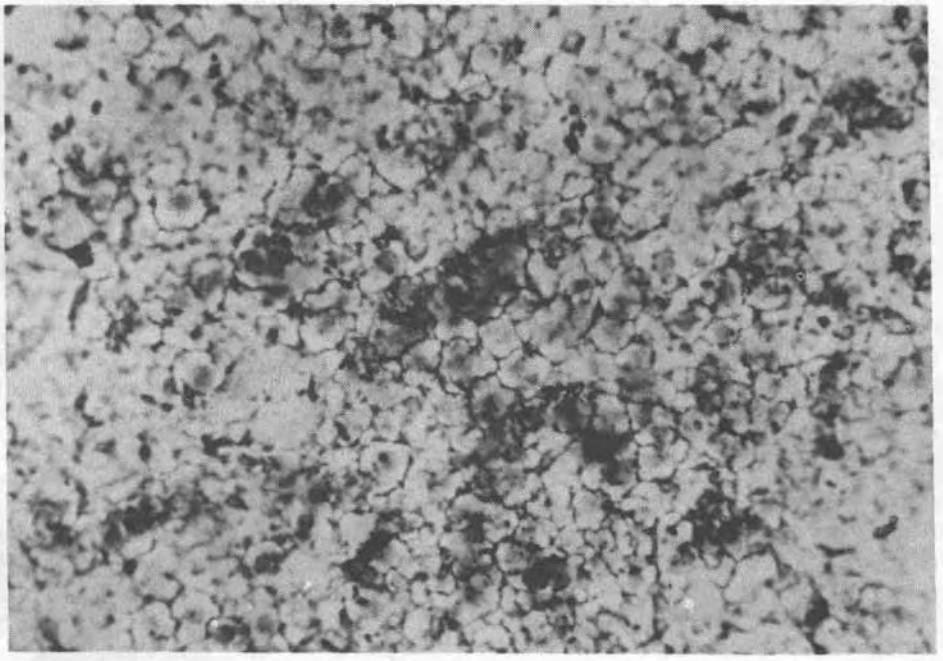

FIG. 3: Frozen section of bone marrow stained with reticulin (x 400). Note the increased reticular network in a case of polycythaemia rubra vera.

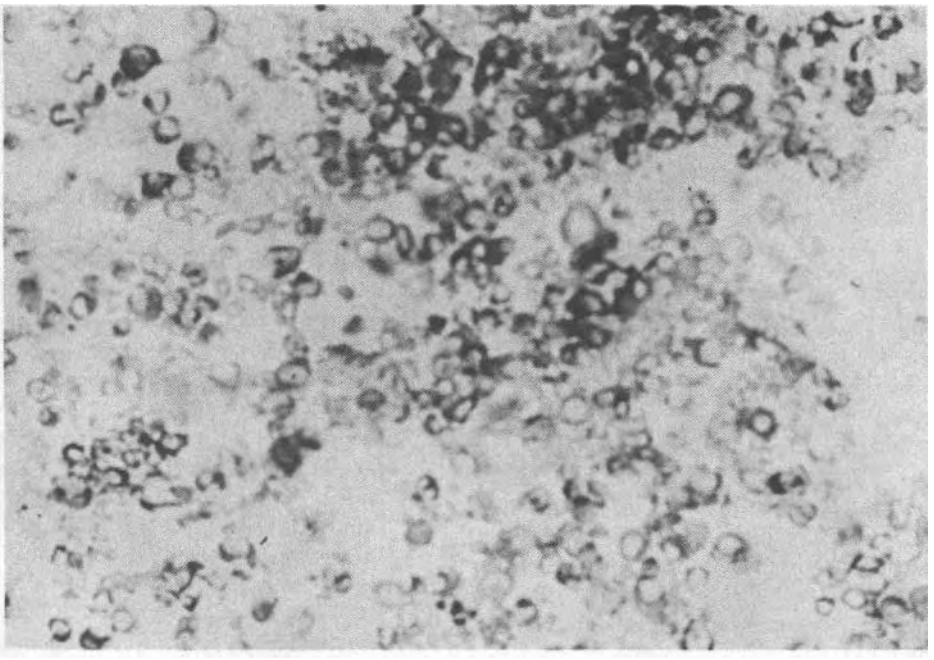

FIG. 4: Frozen section of bone marrow stained with immunoalkaline phosphatase technique using monoclonal antibodies CD45 (x 400).

such as alcohol and toluene as in the conventional paraffin technique. We also observed that the microtome knife was not damaged by the procedure.

Poor localisation of enzyme activity to the cells was sometimes observed especially when the slides were stored. This can be prevented by wrapping in aluminium foil or brief fixation in neutral 10% calcium-formalin before storing at −20°C. Good localization of alkaline phosphatase was demonstrated in aluminium-wrapped sections after one year.

Fragmentation of the trephine specimen sometimes occurred during sectioning especially when the biopsy core had been taken near the cortex of the bone. This can be largely prevented by taking biopsies from the medulla. This problem has also been minimised with the introduction of the gum-sucrose step which reduces fragmentation of the specimens and tearing around the bony trabeculae. A well-chilled and newly-sharpened microtome knife will also minimize fragmentation.

One disadvantage of the frozen section technique is the fading of various stains on storage. At the time of writing, sections stained with H & E, Leder-Giemsa, reticulin, alkaline phosphatase and S'nucleotidase have not faded after two and a half years. However, sections stained with peroxidase, dual esterase, acid phosphatase and immunoalkaline phosphatase have faded. These latter stains only last 3 to 6 months.

In conclusion, the cryostat technique can be an invaluable tool in the routine study of bone marrow biopsies. It can supplement, if not replace, the traditional paraffin technique.

REFERENCES

1. Galton DAG. Foreword. In: Bartl R, Frisch B, Burkhardt, eds. Bone marrow biopsies revisited. Basel, New York: Karger, 1985.
2. Trubowitz S, Davis S. The human bone marrow: anatomy, physiology and pathophysiology. Volume I. Boca Raton, Florida: CRC Press Inc., 1982: 1–14.
3. Frisch B, Lewis SM, Burkhardt R, Bartl R. Biopsy pathology of bone and bone marrow. London: Chapman and Hall, 1985: 1–17.
4. Burkhardt R, Frisch B, Bartl R. Bone biopsy in haematological disorders. J Clin Pathol 1982; 35: 257–84.
5. Blazek J, Georgii A. Histochemical and immunohistochemical examination of plastic sections. In: Lennert K, Hubner K, eds. Pathology of the bone marrow. Stuttgart; New York: Fischer, 1984: 18–20.
6. Beckstead JH, Halverson PS, Ries CA, Bainton DF. Enzyme histochemistry and immunohistochemistry on biopsy specimens of pathologic human bone marrow. Blood 1981; 57: 1088–98.
7. Taylor CR. Immunohistologic studies of lymphoma. Past, present and future. J Histochem Cytochem 1980; 28: 777–87.
8. Chilosi M, Pizzolo G, Janossy G, Bofill M, Fiore-Donati L. Enzyme histochemical analysis on cryostat sections of human bone marrow. J Clin Pathol 1982; 35: 1220-6.
9. Bartl R. Frozen sections of undercalcified bone marrow biopsies. In: Lennert K, Hubner K, eds. Pathology of the bone marrow. Stuttgart; New York: Fischer, 1984: 21–2.
10. Bankcroft JD, Steven A. Theory and practice of histological techniques. Churchill Livingstone, 1982: 89.
11. Cordon H, Sweets HH. A simple method for silver impregnation of reticulum. Am J Pathol 1936; 12: 545.
12. Li CY, Yam LT, Crosby WH. Histochemical characterisation of cellular and structural elements of the human spleen. J Histochem Cytochem 1972; 20: 1049–58.
13. Goldberg AF, Barka T. Acid phosphatase activity in human blood cells. Nature 1962; 195: 297.
14. Rutenberg AM, Rosales CL, Bennett JM. An improved histochemical method for demonstration of leukocyte alkaline phosphatase activity: clinical application. J Lab Clin Med 1965; 65: 698.
15. Silber R, Conklyn M, Grusky G, Zucker-Franklin D. Human lymphocytes: S'nucleotidase – positive and negative subpopulation. J Clin Invest 1975; 56: 1324–27.
16. Cheong SK, Chong SM. Leder-Giemsa stain for trephine biopsy – a technical study. Malays J Pathol 1986; 8: 75–9.
17. Kaplow LG. Simplified myeloperoxidase stain using benzidine dihydrochloride. Blood 1965; 26: 215–9.
18. Sun T, Li CY, Yam LT. Atlas of cytochemistry and immunochemistry of hematologic neoplasms. Chicago: American Society of Clinical Pathologists, 1985: 132.