SECTIONING OF BONE MARROW ASPIRATES: A COMPLEMENTARY APPROACH TO BONE MARROW SMEARS FOR DIAGNOSING HAEMATOL OGICAL DISORDERS

Saneya Kamel and Nour El Hoda Ismail

From Clinical Pathology and Pathology Departments, Faculty of Medicine, Cairo University

ABSTRACT

Bone marrow aspirates were taken at diagnosis from fifty patients with different haematological disorders. Fourteen patients with normal bone marrow were used as a control group.

Sectioning of marrow particles using a simple method was described. Results were studied together with the peripheral blood and bone marrow smears.

Excellent histological preparations of marrow, showing the general architecture, the proportion of fat and cellular tissue, the number and disposition of the megakaryocytes, iron deposits in reticuloendothelial cells and erythroblasts were obtained.

In idiopathic thrombocytopenic purpura (ITP) cases, abnormal magakaryocytes were more easily seen in sections rather than smears. In aplastic anaemia, the marrow sections showed the haemopoietic elements indicating a true sample of the marrow. Malignant infiltration of bone marrow was detected only in marrow sections, while peripheral blood and bone marrow smears failed to indicate the diagnosis. In leukaemic cases, smears were superior to sections due to better discrimination of normal haemopoietic and leukaemic cells specially with minimal infiltration and during chemotherapy. However, marrow sections in the other cases studied revealed and confirmed the same achievement obtained by marrow smears.

It is concluded that a satisfactory evaluation of marrow samples for diagnostic studies can be achieved by examination of smears along with sections of aspirate particles. Sectioning of marrow particles is particularly important for identifying metastatic infiltration of bone marrow. It is recommended to be used in all cases as a supplement to the usual smear technique.

INTRODUCTION

The value of bone marrow puncture as a diagnostic measure in disorders of the haemopoietic system is widely accepted by haematologists, and the procedure is generally recognized as being safe and causing little or no discomfort to the patient (Capeli et al., 1947).

The usual method of dealing with the aspirated material consists of the microscopical examination of marrow smears stained by one of the Romanowsky stains, a differential count of the nucleated cells, and an estimation of leucocytogenic-erythrogenic ratio. Other workers carry out total nucleated cell counts and supravital preparations may be made if needed (Berman), 1953). These procedures, while of great value cytologically, do not always give a true picture of the marrow as a whole and give no indication of its architectural arrangement (Kuper, 1965; Dee et al., 1976 and Joannides and Rywlin, 1976).

A simple method of preparing good histological sections from sternal marrow aspirates would be of great service and the sections would yield valuable additional information about the architecture of the marrow (Pasquale and Chikkappa, 1986).

Many haematologists and pathologists do not routinely examine sections of bone marrow particles. This seems to be due in part to the relative complexity or tediousness of the technique requiring anticoagulants, special equipment or biopsy needles (Berman, 1953; Raman, 1955 and Rywlin et al., 1970).

This paper purposes to give a short account of the method adopted for preparing satisfactory marrow sections from marrow puncture material at the bed side or in the laboratory and its use in clinical practice.

MATERIAL AND METHODS

This study included the bone marrow aspirates of the following disorders:

I.Control group comprised 14 patients having normal bone marrow. They were admitted to hospital for cold operations (neurofibroma of the left thigh, 3 cases with undescended testis, inguinal hernia, lipoma over right shou der, 4 cases with paraumbilical hernia and 4 cases with hydrocele. The bone marrow samples were collected in the theatre while the patients were under general anaesthesia.

II. Patients group consisted of 50 patients as the following:

* 9 patients with ITP,
* 19 patients with different types of anaemias:
  - 5 patients with aplastic anaemia.
  - 7 patients with anaemia of chronic disorders.
  - 4 patients with magaloblastic anaemia, and
  - 3 patients with iron deficiency anaemia.
* 15 patients with acute leukaemia,
* 3 patients with malignant infiltration of bone marrow (neuroblastoma, cancer breast and cancer prostate),
* 3 patients with hypersplenism, and
* 1 case of benign eosinophilic hyperplasia.

Bone marrow aspirate and a sample of peripheral blood were taken from each patient. The aspirate was collected and dealt with as follows:

* After adequate local anaesthesia has been obtained and with due aseptic precautions, marrow is aspirated. A 16 or 18 gauge bone marrow needle yields good particles. After introduction of the needle into the bone, the stylet is withdrawn and a 20 ml plastic syringe is attached. The plunger of the syringe is pulled back vigourously and about 0.25-0.5 ml of marrow is aspirated. Strong suction should be applied when the marrow cavity is entered in order to aspirate

actual fragments of the tissue and to avoid undue admixture with blood which tends to spoil the films.

⋆ A dozen of thin films are made by spreading a minute drop of the marrow on microscopic slides. Two smears containing good particles were squashed then stained with the others with Leishman's stain (Dacie and Lewis, 1984) and Perl's prussian blue stain (Douglas and Dacie, 1953).

⋆ The remainder of the marrow is ejected into a 20 ml tube containing 10% formol saline as a fixative for at least one hour followed by the following steps:

- Centrifuge to get the deposit.
- Take for processing by taking the particles covered in filter paper.
- Embed the particles in paraffin wax and make cell block in the usual way.
- Cut sections from the block (thin sections, 3-5 microns).
- Stain in haematoxylin and eosin (Mc Mannus and Mowr y, 1960 and Perl's prussian blue (Pearse, 1970)
- Mount in DPX mounting media then examine under the microscope.

RESULTS

Excellent histological Preparations of marrow are obtained which show the general architecture, the proportion of fat and cellular tissue, the relationship of leucopoietic and erythropoietic tissues to one another and to the supporting framework. The number and disposition of the megakaryocytes are readily assessed and the presence of abnormal elements, as shown in ITP (fig.1) is more easily detected than in the smear preparations. we have found that, unless large numbers of smears are examined, a true impression of the marrow may not be obtained specially in regard to the megakaryocytes which are not usually evenly dispersed.

In case of aplastic anaemia, the marrow sections in the five cases studied remove all doubt as to whether the lack of haemopoietic elements in smears is due to failure to obtain a true sample of the marrow (fig.2).

Abnormal elements and metastatic infiltration are recognized in the sections and not in smears even in squashed smears in the three cases studied (i.e., neuroblastoma, cancer breast and cancer prostate and confusion due to admixture of blood does not arise (fig.3)

The smear preparations in leukaemic cases were superior to sections of aspirate particles as a result of better discrimination of normal haemopoietic and leukaemic cells specially with minimal infiltration and during chemotherapy.

In the other cases studied in this work, both sections and smears revealed the same result.

Regarding Parl's prussian blue stain, it seems that it did not add much to the result as both sections and smears gave the same content of iron deposits in both reticuloendothelial cells and erythroblasts. However, it is necessary to stain both preparations with prussian blue to compare both results as different results may be obtained due to technical changes. The latter observation was sometimes noticed in patients with anaemia of chronic disorder where iron was obtained in sections only while smears were supposed to have some in reticuloendothelial cells.

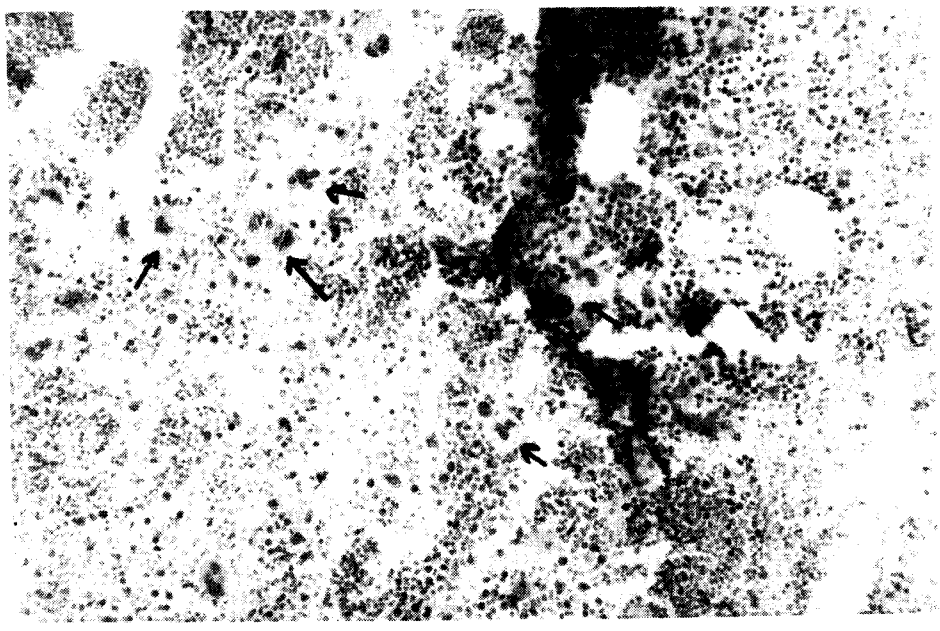


Fig.(1): Histologic section of bone marrow particles showing considerable number of megakaryocytes stained with Hx.& E.

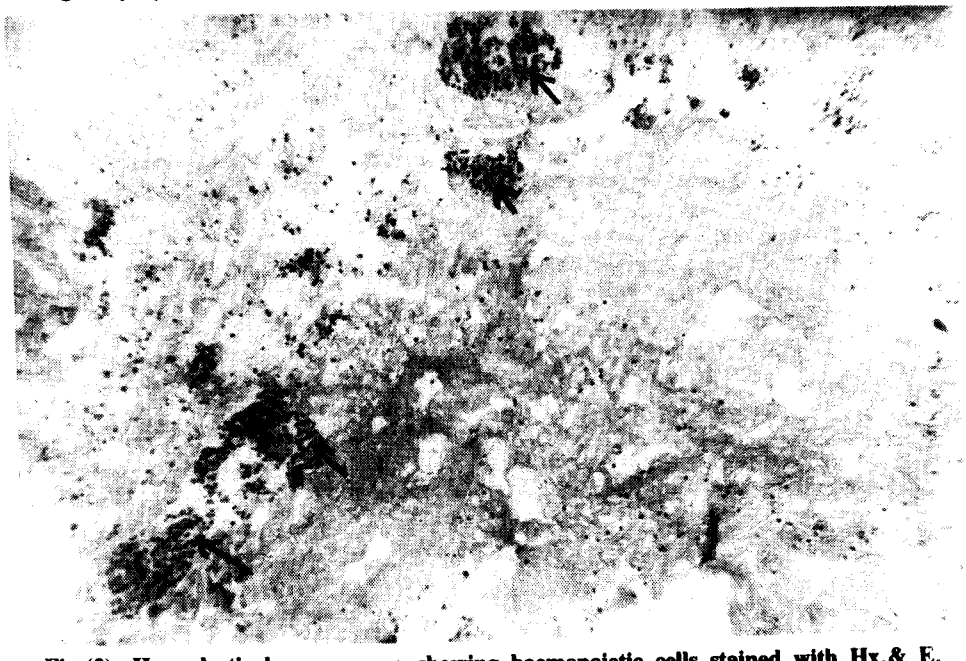


Fig.(2): Hypoplastic bone marrow showing haemopoietic cells stained with Hx.& E.

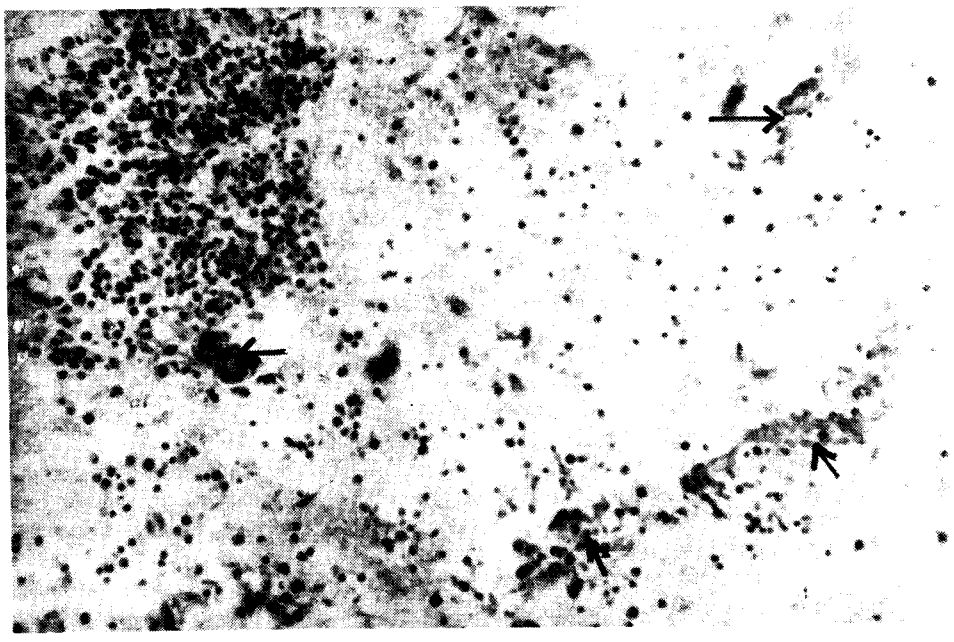


Fig.(3): Metastatic infiltration of bone marrow stained with Hx.& E.

DISCUSSION

Since surgical removal of marrow for the purpose of preparing sections is attended by unneccessary inconvenience to the patient, it is important that complete use be made of the material obtained by simple aspiration.

It is the aim of this study to prepare satisfactory marrow sections from marrow aspirates and find out its importance in clinical practice.

Tissue sections made from the small particles of aspirated marrow are particularly valuable for the detection of tubercles, granulomas, tumours and other structures not usually seen in smears or in prints (Berman, 1953).

Some investigators recommended the simple method of allowing a portion of the marrow material to clot with its admixture of blood and particles. Apart from the fact that the blood surrounding the particles interferes with rapid fixation, an additional serious practical difficulty is that this method requires too much of the time of a tissue technician, who has to make serial sections of the entire clot in order to cut through all the fragments of marrow present at different levels (Miller and trubowitz, 1968).

Rywlin et al. (1970) recommended a method which yielded a better histologic preparation than clot section of bone marrow. They demonstrated that histologic sections of aspirated bone marrow particles are superior in some ways to those prepared from specimens obtained by needle biopsy. The latter often show crushing artifacts and require decalcification which results in decreased cytologic detail. They resorted to needle biopsy of

the bone marrow only in cases of a dry tap or when there was an indication that bone histology should be studied in addition to the marrow. The study of Dee et al. (1976) demonstrated that lymphomatous involvement of bone marrow using trephine biopsy was superior to aspiration in most instances specially for the nodular form. In some cases, examination of paraffin embedded clot section and smears was diagnostic of lymphoma whereas the trephine biopsy section appeared negative.

Furthermore, Loannides and Rywlin (1976) compared histologic sections of bone marrow obtained by aspiration and by needle biopsy. They found that more marrow was secured and larger areas of marrow were seen in aspirated than in biopsied specimens. Cytologic details were easier to appreciate in aspirates than in biopsies. Giemsa stain were far better in aspirates than in the decalcified biopsies. Bone morphology could be observed in biopsies only.

In 1976, Rywlin suggested that one should not conclude that needle biopsy is superior to histologic examination of aspirated marrow. The ultimate decision whether needle biopsy is superior to aspiration awaits controlled comparison between needle biopsy and an adequate aspiration technique.

In our study, the results indicate that sections of bone marrow particles complement the findings of bone marrow aspiration in the diagnosis of hypoplastic marrow, ITP and marrow infiltration by secondaries (which may be difficult to assess by the smears alone).

Comparative evaluation of bone marrow aspirate particles smears, biopsy imprints and biopsy sections were done by Pasqual and Chikkappa (1986). They concluded that any of the three marrow preparations alone is not sufficient for accurate diagnosis in all cases.

When peripheral blood and bone marrow aspirate samples fail to indicate the diagnosis, a long core biopsy may yield positive results. This suggestion was made when a group of patients suffering from acute myeloid leukaemia, chronic granulocytic leukaemia and acute lymphoblastic leukaemia was studied (Islam and Henderson, 1988).

Im et al. (1988) investigated the necessity to evaluate both bone marrow aspirates and biopsy together in malignant lymphoma.

Lately, the work done by Islam et al. (1989) demonstrated that plastic embedded bone marrow biopsy sections complement the findings of bone marrow aspiration in the diagnosis of acute myeloid leukaemia and may provide information of independent prognostic importance that cannot be obtained by other means.

In this study, the smear preparations in leukaemic cases were superior to sections of aspirate particles as a result of better discrimination of normal haemopoietic cells and leukaemic cells specially with minimal infiltration and during chemotherapy. These results were in accordance with Dee et al. (1976) who investigated a group of patients suffering from Hodgkin's and non-Hodgkin's lymphoma.

It is true that in the majority of cases, simple marrow smears are enough for diagnosis, but as the haematologist's experience with bone marrow examination enlarges, he or she will sooner or later be confronted with problems that cannot be solved by examination of smears alone. The routine procedure mentioned in this study may seem to make an elaborate task of what is thought to be very simple matter of examining both sections and smears of aspirated bone marrow particles.

In conclusion, a satisfactory evaluation of marrow samples for diagnostic studies can be achieved by examination of

smears along with sections of aspirate particles. Any of the two marrow preparations alone is not sufficient for accurate diagnosis in many cases. Section of marrow particles when carried out is an accurate modality for identifying nonhaematological tumour metastasis in the bone marrow and can add much to the service of the laboratory as complete use is to be made of the material obtained by simple aspiration.

Further investigation to study sectioning of marrow particles versus marrow biopsy is recommended to clarify the role of each procdure in solving haematological problems.

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قطاعات من شفط نخاع العظم : طريقة مكملة لمسحة نخاع العظم فى تشخيص أمراض الدم

سنية كامل ونور الهدى اسماعيل

يهدف هذا البحث الى عمل قطاعات من شفط نخاع العظم بطريقة مبسطة وعلاقتة بطريقة مسحة النخاع كمحاولة للاستفادة بطريقة كاملة من شفط النخاع ومعرفة اهميتة فى الممارسة الاكلينيكية .

اجرى البحث على ٦٤ حالة قسمت الى مجموعتين :

المجموعة الضابطة (١٤ حالة) ومجموعة المرضى (٥٠ حالة) مصابين بامراض الدم المختلفة .

ظهرت عينات النخاع الهستولوجية بصورة ممتازة موضحة التركيب العام ، ونسبة الدهون والنسيج الخلوى فى عدد وترتيب الخلايا النقيبة الكبيرة (الميجاكاريوسيت) ترسيب الحديد فى الخلايا الملهمة والخلايا البدائية الدموية (الارثروبلاست) وفى الحالات المرضية ظهرت الميجاكاريوسيت الغير طبيعية فى القطاعات اكثر وضوحا من المسحات وكان هذا واضحا فى حالات نقص الصفائح الفرفيرية المجهول السبب وفى حالات فقر الدم الناتج عن توقف النمو ظهرت خلايا الدم البدائية فى القطاعات موضحة صحة عينة النخاع وليس فشلا فى سحب العينة الشىء الذى يوفر على المريض اعادة البذل . وفى حالات لوكيميا الدم اتضح ان مسحات نخاع العظم كانت متفوقة على القطاعات ويرجع ذلك الى التفرقة الجيدة لخلايا الدم السليمة والسرطانية وخاصة فى حالات التغلغل البسيط واثناء العلاج الكيميائى . مع ذلك فقطاعات حبيبات النخاع فى الحالات الأخرى من البحث ظهرت واكدت نفس النتيجة التى حققتها مسحات النخاع .

يستنتج من هذا البحث ان التقييم الكافى لعينات النخاع فى دراسات التشخيص يمكن تحقيقة بفحص المسحات بجانب قطاعات حبيبات الشفط وان قطاعات النخاع ذات اهمية كبرى فى التعرف على التغلغل السرطانى لنخاع العظم ، ويوصى باستعمالها فى جميع الحالات كطريقة مكملة لطريقة المسحات التقليدية علاوة على انها طريقة بسيطة يمكن بها الاستعمال الكامل لشفط النخاع فلا تبديد فى المادة الثمينة التى يحصل عليها احيانا بصعوبة . وقد تضيف كثيرا للتشخيص دون ادنى ضرر للمريض .