

Paraffin embedded marrow clot sections as an adjuvant procedure in the diagnosis of bone marrow diseases.

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ABSTRACT

Objectives: This study was conducted to evaluate the role of histopathological examination of paraffin embedded marrow clot sections in the diagnosis of different neoplastic and non-neoplastic marrow diseases, and whether these sections can replace the need for performing bone marrow trephine biopsy.

Methods: Patients included in the study are those with clinical manifestations of hematological disorders who were subjected to peripheral blood examination, marrow aspirate cytology with complimentary bone marrow trephine and marrow clot biopsy. Along with marrow aspiration, the blood left behind after preparing marrow smears, was used to prepare paraffin embedded histological clot sections. The results for 114 patients were analyzed simultaneously for their concordance to highlight the usefulness of marrow clot sections in the diagnosis of different diseases affecting the bone marrow. Immunohistochemical stains were used whenever indicated to obtain additional diagnostic information.

Results: Marrow clot sections were diagnostic and concordant with the complete blood count, marrow aspirates and trephines in 63% of the cases. While trephines were unconvincing in another 15.7% of the cases, the clot sections were diagnostic. In 1.7% of the cases, the clot sections and trephines were diagnostic while marrow aspirates were inconclusive, thus in 81% of the cases, marrow clot sections provided the diagnosis and replacing the need for performing trephine biopsy. However, trephine biopsy is still considered necessary in 19 % of the cases in whom the clot sections are of poor quality and unconvincing

Conclusion: In most of the cases clot sections can replace the need for trephine biopsy, but trephine biopsy is still required in other cases. The clot sections are useful as an adjuvant procedure to increase the diagnostic yield in marrow studies. Because it is difficult to expect which patients will be diagnosed by clot sections and which will need trephine biopsy, it is advisable to perform both trephine and clot biopsy together with the marrow aspirates.

Keywords: Bone marrow trephine biopsy, marrow clot biopsy, marrow aspirates

فحص المقاطع النسيجية لخثرة نخاع العظم كطريقه مساعده لتشخيص امراض نخاع العظم

أهداف البحث: أجريت هذه الدراسة لتقييم دور فحص المقاطع النسيجية لخثرة نخاع العظم في تشخيص مختلف امراض

النخاع الورمية وغير الورمية، وما إذا كانت هذه المقاطع النسيجية يمكن أن تغني عن الحاجة لإجراء خزعة نخاع العظم.

طريقة العمل: المرضى المشمولين في هذه الدراسة هم اللذين يعانون من اختلالات دموية والذين اجريت لهم فحوصات الدم

المحيطي والفحص الخلوي لسحب خلايا النخاع مع خزعة نخاع العظم وخزعة خثرة النخاع. بعد سحب النخاع وتحضير

مسحات الفحص الخلوي للنخاع تتم الاستفادة من الدم المتبقي لتحضير مقاطع نسيجية من خثرة الدم. تم تحليل نتائج 114

مريض في وقت واحد لتطبيقها مع بعضها وصولاً للتشخيص النهائي وذلك لتسليط الضوء على فائدة فحص المقاطع النسيجية

لخثرة النخاع في تشخيص الأمراض المختلفة التي تصيب نخاع العظم. كما استخدمت الاصبغ المناعية النسيجية حسب الحاجة للحصول على معلومات تشخيصية إضافية.

النتائج: كانت نتائج فحوصات المقاطع النسيجية لخثرة نخاع العظم مشخصة ومتطابقة مع تعداد الدم الكامل والفحص الخلوي لسحب خلايا النخاع وكذلك مع خزعة نخاع العظم في ٦٣٪ من الحالات. في حين كانت خزعة نخاع العظم غير مقنعة في ١٥.٧٪ من الحالات كانت المقاطع النسيجية لخثرة النخاع مشخصة. وفي ١.٧٪ من الحالات، كانت المقاطع النسيجية لخثرة النخاع وخزعة نخاع العظم مشخصة بينما كانت مسحات الفحص الخلوي للنخاع غير حاسمة. وهكذا فإنه في ٨١٪ من الحالات، كانت المقاطع النسيجية لخثرة النخاع تشخيصيه مع امكانية الاستغناء عن الحاجة لأداء خزعة نخاع العظم. ومع ذلك فإن خزعة نخاع العظم كانت ضرورية للتشخيص في ١٩٪ من الحالات التي تكون فيها المقاطع النسيجية لخثرة النخاع ذات نوعية رديئة وغير مقنعة.

الاستنتاج: في معظم الحالات يمكن للمقاطع النسيجية لخثرة النخاع ان تحل محل خزعة نخاع العظم، ولكن ما زالت هناك حاجة لعمل خزعة نخاع العظم في حالات أخرى. تعتبر المقاطع النسيجية لخثرة النخاع مفيدة كإجراء مساعد لزيادة الغلة التشخيصية في فحوصات نخاع العظم وبما أنه من الصعب أن نتوقع أي من المرضى سيتم تشخيصهم عن طريق المقاطع النسيجية لخثرة النخاع وأبهم سيحتاج الى خزعة نخاع العظم، فإنه من المستحسن عمل خزعة نخاع العظم وخزعة نخاع النخاع جنباً إلى جنب مع الفحص الخلوي لسحب خلايا النخاع

INTRODUCTION

Bone marrow examination is useful in the diagnosis of various hematological diseases. Both neoplastic and non-neoplastic hematological diseases can be diagnosed with a maximum diagnostic yield by the simultaneous examination of peripheral blood, marrow aspirate smears, marrow trephine and clot biopsy sections.^[1-3] The blood clot collected during marrow aspiration can be used to prepare paraffin embedded histological sections. With the use of immunohistochemical stains, marrow clot sections may provide an adjuvant procedure to improve the diagnostic yield in different neoplastic and non-neoplastic marrow diseases. Studies, worldwide, have been done to evaluate the role of marrow clot sections as an adjuvant diagnostic method in different marrow diseases.^[2-6] However, this is the first study on marrow clot sections to be conducted in our locality to evaluate the role of paraffin embedded marrow clot sections in the diagnosis of different marrow diseases, and

whether these sections can replace the need for performing bone marrow trephine biopsy.

MATERIALS AND METHODS

This is a prospective study, performed in Basrah governorate-Iraq, during the period from January 2013 through April 2015. A 114 patients, sixty five males and forty nine females, all are those with clinical manifestations of hematological disorders who were subjected to peripheral blood examination, marrow aspirate cytology with complimentary bone marrow trephine and marrow clot biopsy. Along with marrow aspiration, 0.5-2 ml of the aspirated blood (the blood left behind after preparing marrow smears) was fixed in 10% formal saline overnight. Trephine biopsy was fixed in Bouin's solution overnight. If more decalcification is required, trephine biopsy is further decalcified with 20% HCL for 1-3 hours. Unnecessary prolonged decalcification of trephine biopsies with acid was avoided to insure good staining

quality. Over fixation of marrow clot biopsies was avoided as this may make the clot brittle with subsequent difficult sectioning. The trephine and clot biopsies are then processed through alcohol, xylene, impregnation in paraffin wax and blocking. Sections (3-5 µm thickness) were stained by Hematoxylin and Eosin stains. Immunohistochemical stains were used as indicated. Clinical data and laboratory findings, including complete blood count, marrow aspirate cytology and results of bone marrow trephine and clot sections for each patient were recorded in an Excel sheet for subsequent analysis.

RESULTS

A total of 114 patients were included in this study. Sixty five (57.1%) were males and forty nine (42.9%) females. The mean age was 51.7 year. The commonest indication for marrow study was anemia (51.7%). Backache due to vertebral destructive lesion was the indication for marrow study in 15.7% of the cases, (Table-1)

Table 1. Indications for bone marrow study

Indications	No.	(%)
Anemia	59	(51.76)
Backache	18	(15.79)
Lymphadenopathy	12	(10.53)
Splenomegaly	11	(9.65)
Non Hodgkin’s lymphoma	4	(3.51)
Hodgkin’s lymphoma	2	(1.76)
Plasma cell myeloma	2	(1.76)
Fever	2	(1.76)
Ecchymosis	2	(1.76)
Thalassemia	1	(0.88)
Sickle cell disease	1	(0.88)
Total	114	(100%)

The final diagnosis was made by the concordance of the results of complete blood count (CBC), marrow aspirate smears, trephine biopsy and marrow clot biopsy (Table-2).

Table 2. The diagnosis of the patients

Diagnosis	No.	(%)
Normal marrow	64	(56.14)
Marrow hypoplasia	9	(7.90)
Aplastic anemia	5	(4.39)
Myelodysplastic syndrome	5	(4.39)
Plasma cell myeloma	4	(3.51)
Non Hodgkin’s lymphoma	4	(3.51)
Chronic myeloid leukemia	4	(3.51)
Chronic lymphocytic leukemia	3	(2.64)
Megakaryocytic hyperplasia	3	2.64)
panmyeloid hyperplasia	3	(2.64)
Acute myeloblastic leukemia	2	(1.76)
Essential thrombocythemia	2	(1.76)
lymphoid hyperplasia	2	(1.76)
Myelofibrosis	1	(0.88)
Megaloblastic anemia	1	(0.88)
Acute lymphblastic leukemia	1	(0.88)
Metastatic carcinoma	1	(0.88)
Total	114	(100%)

Marrow clot biopsy was performed for all patients included in this study. Conclusive and diagnostic marrow clot sections were obtained in 92 out of 114 cases (80.7%). In 72 of these 92 cases (63% of total) the clot sections yielded results that are concordant with those of the trephine biopsy, marrow aspirate smears and complete blood count. In 18/92 cases (15.7% of total), the trephine biopsies were inconclusive, composed of bone trabeculae only, while marrow clot sections were conclusive, yielded results that are concordant with the marrow aspirate smears and complete blood count.

Furthermore, in 2/92 cases (1.7%) the clot sections were concordant with trephine biopsy while marrow aspirate smears were inconclusive. Poor quality, inconclusive,

marrow clot samples were demonstrated in 22 cases (19.2%), composed of blood clot only or few scattered hematopoietic cells (table-3)

Table 3. Results of clot sections examination in comparison with other procedures

			No.	(%)	Clot+	
					No.	%
Clot+	Asp+	Treph+	72	63.16	92	80.7
Clot+	Asp+	Treph-	18	15.79		
Clot+	Asp-	Treph+	2	1.76		
Clot-	Asp-	Treph+	2	1.76		
Clot-	Asp+	Treph+	12	10.53		
Clot-	Asp+	Treph-	8	7		
Clot-	Asp-	Treph-	0	0		
Total			114	100		

(+) = conclusive (-) = inconclusive

Asp=marrow aspirate smears, Clot = clot sections, Treph = trephine biopsy.

DISCUSSION

The role of histopathological examination of paraffin embedded marrow clot sections has been evaluated as an additional diagnostic method in different neoplastic and non-neoplastic diseases affecting the bone marrow. However, limitations of marrow clot sections have been noted in these works.^[2-6] In this study, marrow clot sections are proved to be diagnostic in 80.7% of cases. In 63% of the cases, clot sections yielded results that are concordant with that of complete blood count, trephine biopsy and marrow aspirate smears. In 17.5% of the cases the trephine biopsy was inconclusive, while the clot sections yielded results that are concordant with those of marrow aspirate smears and complete blood count. Tkachuk and Hirschmann concluded that marrow clot sections can be conclusive while trephine biopsy and marrow aspirate cytology failed to show any pathology.^[4] Dee et al found that , in some cases, marrow clot sections and smears are diagnostic of lymphoma whereas the trephine biopsy appeared negative.^[9] Similarly, Mathur and Soutar in their work on the role of marrow clot sections in confirming marrow involvement by multiple myeloma, reported that “the clot sections provided the diagnosis in 75% of the patients (19/28) and in five of these

19(26%) the trephine biopsy was negative”.^[7] This highlights the necessity for preparing marrow clot sections whenever bone marrow study is performed, particularly when trephine biopsy is obtained with difficulty. Furthermore, in immunohistochemical staining, antigen retrieval is better in clot sections than trephine biopsy since decalcification is not required.^[5] Decalcification has the disadvantage of damaging nucleic acids and proteins reducing the staining quality.^[8] As compared with trephine biopsy, the clot biopsy saves time, avoids discomfort, reduces cost with less incidence of complications.^[7] This study showed that in most of the cases (80.7%) the clot sections can replace the need for trephine biopsy. However trephine biopsy is still required in other cases, in whom the clot biopsies are of poor quality and inconclusive. This is in agreement with other studies^[5,7] Ong et al has concluded that “a bone marrow section comprised of sinusoidal blood is inadequate for morphological interpretation”, the authors recommend the “cell block technique to ensure maximum capture of material needed to establish a diagnosis”.^[6] Because it is difficult to anticipate which patients will be diagnosed by the clot sections, and which will need

trephine biopsy, it is advisable to perform both trephine and clot biopsy together with the marrow aspirate. The clot biopsy is prepared as an adjunct procedure to increase the diagnostic yield.

CONCLUSION

Examination of marrow clot sections in conjunction with the complete blood count, marrow aspirate smears and trephine biopsy is necessary to improve the diagnostic yield of these procedures. In most of the cases clot sections can replace the need for trephine biopsy, but trephine biopsy is still required in other cases.

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