

Original article

Evaluation the Role of Bone Marrow Examination in Diagnosis of Hematological Diseases in Hemato-Oncology Centers in Kurdistan Region

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Abstract

Background: Bone marrow aspiration and trephine biopsy have an important role in evaluation and diagnosis of most hematological and some non hematological disorders.

Objectives: The aims of this study were to assess the value of bone marrow examination in diagnosis of hematological diseases and determination of frequency and causes of dry tap marrow.

Patients and Methods: This study was prospective only and it was conducted from January 2013 to June 2013 at Hiwa and Nanakaly hospitals in Sulaymaniyah and Erbil respectively. A total number of 245 cases were underwent bone marrow examination. Touch imprint was prepared in a state of dry tap marrow. Sudan black stain was used in acute leukemia.

Results: The patients comprised 138 (56.3%) males and 107 (43.7%) females, with ages ranging from 1 to 81 years and the mean age of cases was 36.7 ± 24 years. The most frequent clinical finding of patients underwent bone marrow examination were pallor (71.4%), followed by fever (32.2%). The main indications were present of blast cells in peripheral circulation 69 (28.2%), followed by bone marrow assessment for staging of lymphoma 37 (15.1 %). The most common diagnoses encountered were: Acute leukemia 69 (28.2%), Active marrow (negative for lymphoma) 27 (11.1%) and active marrow 24 (9.8%). The frequency of dry tap was (4.08%) and the most common causes were: acute leukemia and faulty technique (normal marrow).

Conclusion: Bone marrow examination is an important diagnostic tool in the diagnosis and staging of various hematological disorders.

Keywords: Bone Marrow Aspiration, Trephine Biopsy, Hematology, Kurdistan

Introduction

Bone marrow is a quite cellular connective tissue that occupying the spaces between bony trabeculae. The main hemopoietic tissue is bone marrow and it accounts for about (5%) of all body weight. Bone marrow aspiration and trephine biopsy have an important role in evaluation and diagnosis of most hematological and some non hematological disorders. Bone marrow examination is needed for staging of lymphoproliferative disorders. In advanced stage, it shows bone marrow involvement. Also trephine biopsy is more important than marrow aspiration in evaluation of lymphoma and metastatic solid tumors. These procedures are valuable in the diagnostic work up of pyrexia of unknown origin; it may shows granuloma, infection, necrosis and hemophagocytic syndrome. They are useful in follow up evaluation of patients who submit chemotherapy, bone marrow transplantation and other patterns of medical management^(1,2,3).

Bone marrow aspiration and biopsy procedures are complementary to each other and superiority of one procedure over the other relied on the particular disorders ⁽¹⁾. In obtaining bone

marrow examination, there are some contraindications which are related to the general condition of the patient. These contraindications are hemophilia, disseminated intravascular coagulation, skin infection and bone disorders such as osteomyelitis or osteogenesis imperfecta ^(4,5).

Both procedures are usually safe but few adverse effects are encountered after bone marrow examination. The popular complication is bleeding which occurs specially in patients with severe low platelet count, or taking aspirin, or receiving anticoagulant as warfarin. Other morbidity from posterior iliac crest are identified and included long standing pain, infection in immune compromised patients and needle-related accidents such as breaking of needle within the bone ^(6,7).

In some certain situation, failure to take out bone marrow on trying marrow aspiration, known as dry tap or blood tap and these are generally described as faulty technique. This difficulty of aspiration may be occurred when histology of marrow is normal. But usually a dry tap indicates important disorders that involve bone marrow. The

most prominent pathological disorders are metastatic carcinoma, myelofibrosis, acute leukemia (packed cells), hairy cells leukemia and lymphoma. In Dry tap status, touch imprint must be prepared for early prime diagnosis before the result of trephine biopsy was returned back ^(8,9). In this study we tried to assess the value of bone marrow examination in diagnosis of hematological diseases and to determine the frequency and causes of dry tap marrow.

Materials and methods

This prospective study has been conducted at Hiwa and Nanakaly hospitals which are only centers for oncology/hematology in Sulaymaniyah and Erbil respectively. All patients underwent bone marrow examination between January 2013 and June 2013, were enrolled in this study. Relapsed and follow-up cases were excluded. A total number of 245 cases were studied. One hundred case was received from Nanakaly hospital and 145 cases were collected from Hiwa hospital.

Inform consent was taken from patient and their relative (In childhood cases). In each case a detailed history with general and systemic examination

and routine investigations (complete blood count, pictures, and erythrocytes sedimentation rate and biochemical tests) were carried out prior to bone marrow examination. The standard technique ⁽⁴⁾ was employed in obtaining the samples from posterior iliac crest by using a biopsy set needle (Modern bone marrow biopsy needle set /11 gauge/ Italian). About 0.5 ml of marrow fluid was obtained and nearly about ten smears prepared. Two slides were stained with Prussian blue technique by well trained staff to demonstrate iron granule and ring sideroblasts when indicated. Sudan Black stain was used routinely for all patients with acute leukemia.

When biopsy was performed, the core biopsy was fixed in formalin and sent for processing in histopathology department (Rizgary Teaching Hospital in Erbil and Shorsh hospital in Sulaymaniyah) and was examined by 2 pathologists independently. Touch imprint smears were usually made in cases with dry tap aspiration.

For some patients with lymphoproliferative disorders and acute leukemia, marrow materials was collected in Ethylene diamine tetra

acetic acid tube and sent for flow cytometry analysis. Also some patients with chronic myeloid leukemia and myeloproliferative neoplasm in whom the diagnosis were not straight forward, specimens were sent for BCR- ABL fusion gene and JAK2 mutation respectively.

The marrow was examined and interpreted by two hematologist and was reviewed with consideration of the patient's clinical and laboratory information. Marrow smears are examined for determination of the cellularity, the morphological details of hemopoietic cells, estimation of myeloid – erythroid ratio and careful search for abnormal cells. A questionnaire was designed that contains a set of questions including (residence, age, sex, clinical features, organomegaly, complete blood picture, other related investigations and bone marrow examination report). The questionnaire type is structured interviewer administrated.

Data were analyzed by using the statistical package for social science (SPSS) version 19. Chi square test of association was used to compare between proportions. A P-value of $\leq$

0.05 was considered statistical significant.

Results

A total of 245 cases underwent bone marrow examination from January 2013 to June 2013 were included in this study. Males were 138 (56.3%) and females were 107 (43.7%). The ratio of male to female was (1.29:1). The age of patients in this project were ranged between 1 to 81 years with mean age of 36.71 ± 24 years.

The indications for bone marrow examination were based on the clinical and or complete blood pictures. In this study, the main indications for examination of bone marrow were presence of blast cells in peripheral circulation which was seen in 69 (28.2%) cases, followed by bone marrow assessment for staging of lymphoma in 43 (17.5%) patients, and then anemia in 20 (8.2%) cases. Leucocytosis was another indication and found in 14 (5.6%) cases, 12 of them showed marked left shift with increased basophilic count and associated with organomegaly, these were diagnosed as CML. The remaining two cases were leucocytosis without

shift to left, one of them was diagnosed as active marrow and the other one regarded as non diagnostic. Other indications were explained in table 1.

Various hematological disorders were encountered in this study as the result of bone marrow examination. The most frequent final diagnosis was acute leukemia and accounting for 69 (28.1%) cases of which 40 patients were diagnosed as ALL and 29 cases were categorized as AML. The next in the descending order of frequency was active marrow (negative for lymphoma) and found in 27 (11.1%) cases. Other final diagnoses are illustrated in table 2.

A comparison was prepared between the indication for bone marrow examination and final diagnosis that made after examination of the bone marrow. Acute leukemias were found in 69 (28.1%) cases and final diagnoses of this category were 40 cases of ALL and 29 cases of AML (fig 1). Of the 37 (15.1%) patients were referred for bone marrow examination for staging of lymphoma, 27 cases didn't showed marrow involvement by lymphoma and 10 cases were demonstrated bone marrow infiltration by lymphoma cells. The next in the descending order of

frequency of indications for bone marrow examination was anemia and was found in 24 (9.8%) patients and the outcome of bone marrow examination were active marrow in (13) cases, multiple myeloma in (7) cases, pure red cells aplasia in (2) cases and erythroid hyperplasia in (2) cases. Table 3

Out of 245 samples 10 (4.08%) cases were regarded as dry taps and touch imprints with trephine biopsy material were obtained from posterior iliac crest for diagnosis. Of these 10 cases, only 3 (30%) patients were revealed active biopsy, while others showed significant marrow pathology. Acute leukemias were the commonest pathological causes of dry taps and found in 3 (30%) cases, two of them were ALL and the remaining one was AML. Other causes of dry tap are clearly explained in (Fig 2).

In total 245 cases were underwent bone marrow aspiration and trephine biopsy no complication were reported.

Discussion

Both bone marrow aspiration and trephine biopsy are essential and safe procedures for diagnosis of various hematological and non-hematological disorders. These are important for follow up of patients who received chemotherapy, or marrow transplantation and/or other medical treatment. Bone marrow aspiration and trephine biopsy are complementary to each other and nowadays, both procedures are performed together and usually on same site routinely ⁽¹⁰⁾.

The most popular indication in this study was presence of blast cells in peripheral circulation (acute leukemia) which were found in 69 (28.2%) cases, followed by staging for lymphoma in descending order of frequency and was found in 37 (15.1%) cases. Similar findings were reported by Bashawri and Al-Gwaiz as acute leukemia was the most common indication and was found in (32.9%) and (26.2%) cases respectively ^(11,12). In contrast to these findings, acute leukemia was ranked fourth common indication (1.25%) in a study done by Bedu-Addo et al in Ghana, in which anemia of unknown

causes was ranked the first frequent indication due to high frequency of nutritional anemia as iron deficiency and megaloblastic anemias¹³. Leucocytosis was also another indication and was seen in 14 (5.6%) patients, where it was mostly due to CML that was found in 12 cases, while the rest two cases were regarded as active marrow and non diagnostic marrow.

The result of this study showed that the acute leukemias were the most frequently encountered diagnosis by bone marrow examination and these were found in 69 (28.1%) cases, 40 patients of them were ALL with mean age 16.8 years and 31 (77.5%) of them were found in 1st and 2nd decades, while 29 cases were AML with mean age 35 years and 10 of them were seen in 3rd and 4th decades; however references set that AML is the commonest acute leukemia in adults and ALL is the commonest childhood malignancy ⁽¹⁴⁾. A similar study was done by Gupta et al and Egesie et al in India and Niger respectively, in which acute leukemias were the largest group disorders ^(15,16). Acute leukemia was ranked third in a

study done by Pudasaini et al from Nepal, in which frequency of erythroid hyperplasia (mostly due to iron deficiency and megaloblastic anemia) were high and these were due to higher prevalence of nutritional deficiency in their locality⁽¹⁷⁾.

In this study, the second common indication for bone marrow examination was staging of lymphoma and found in 37 (15.2%) cases. Similarly, Saeed and Jawhar have also reported that lymphoma were the second common diagnostic report⁽¹⁸⁾. Bone marrow was not involved in 27 (72.9%) cases and marrow involved by lymphoma was found in 10 (27.1%) cases. All specimens of involved marrow were non Hodgkin's lymphoma and the majority of these cases were diffuse large B cell lymphoma. Similar results were reported by Durosinmi et al from Nigeria⁽¹⁹⁾.

Active bone marrow was 3rd common diagnosis in the present study and found in 24 (9.8%) cases. High percentage of active marrow 125 (35.1%) cases was reported in a study from Kenya. These were due to over induction of bone marrow examination

even in cases with bicytopenia and mild anemia⁽²⁾.

ITP was seen in 21 (8.6%) cases in this study. The diagnosis of ITP is made after exclusion of other causes of low platelet count. Other studies were showed 15.7%, 10.5% and 6.21% cases of ITP in Pudasaini et al, Kibria et al^{and} Khan et al respectively^(17,20,21).

Myeloproliferative neoplasms were commonly diagnosed on bone marrow examination and confirmed by estimation of Janus-associated kinase 2 (JAK2) mutations⁽¹⁴⁾. MPN was found in 21 (8.6%) cases. This is nearly similar to another study which was done by Saeed and Jawhar in Mosul¹⁸, while other study was showed low percentage of MPN that found in (2.7%) cases and was published in Yemen⁽²²⁾.

Another common disorder in present study was chronic lymphocytic leukemia and was found in 13 (5.3 %) cases. Similarly, 2 (5%) cases were seen in another study done in India¹⁵. However in a study done by Chandra, 3.1% of cases were CLL²³. This might be related to geographical variation.

Chronic myeloid leukemia was found in 12 (4.9%) cases and was the least common leukemia in studied cases constituting (13%) of all leukemia in this study and it is nearly similar to the fact that CML accounts for around 15% of leukemias⁽¹⁴⁾.

Multiple myeloma was another hematological malignancy that diagnosed by bone marrow examination with other diagnostic criteria and it was found in 12 (4.9%) patients with mean age 61 years and all cases were associated with high ESR. Other studies were reported a frequency of multiple myeloma as 5.6%, 4% and 3.5% respectively^(17,2,24).

Another important indication for bone marrow examination was detection of secondary metastasis. In this study, 8 (3.2%) cases of tumors (4 cases of neuroblastoma, 2 cases of Ewing sarcoma, 1 case of adenocarcinoma of prostate and 1 case of hepatocellular carcinoma) were underwent bone marrow examination to exclude marrow involvement. The marrow involvement by secondary metastasis was found in 2 (0.8%) cases. A study from Lahore reported a similar

finding (0.7% of cases with secondary metastasis)⁽²⁵⁾.

In present study, 6 (2.4%) cases were not diagnosed on bone marrow examination as two of them were with inadequate trephine biopsy and the remaining 4 cases were with inadequate of both aspiration and biopsy. Higher frequency (14.4% cases) of non diagnostic sample was found in another study⁽²⁶⁾.

This study showed that bone marrow aspirations was failed to obtained in 10 (4.08%) cases in which touch imprint and adequate trephine biopsies were well done, this is known as dry tap or blood tap. The main causes of dry tap in present study were faulty technique (active marrow biopsy) in 3 (30%) cases and acute leukemia (packed cells in trephine biopsy) in 3 (30%) cases. A similar study was done by Humphries in 1990, who reported a rate of dry tap as (3.9%)⁹ and higher frequency was recorded by Khanum et al in Lahore and found in 50 (10%) cases among 500 studied patients⁸. The remaining cases of dry tap were myelofibrosis in 2 cases, secondary metastasis and hairy cells leukemia.

In this study, complications after both procedures (marrow aspiration and trephine biopsy) were not encountered. Complications of bone marrow examination are rare as reported by Bain et al⁽²⁷⁾.

Conclusion:

We concluded that bone marrow examination is an important diagnostic tool in the diagnosis and staging of various hematological disorders. The main indication for bone marrow examination in this study was presence of blast cells in peripheral circulation (Acute leukemia). The commonest causes of dry tap in this study were faulty technique and packed cell in acute leukemia. Bone marrow examination in spite of being invasive procedure it is safe and post operative complications were not recorded in this study.

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تقييم دور فحص نخاع العظم في تشخيص أمراض الدم في مراكز أمراض الدم و الأورام في إقليم كردستان

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الملخص

الخلفية: لعينة نخاع العظم والخزعة دورا هاما في تقييم وتشخيص معظم أمراض الدم وبعض الاضطرابات غير الدموية. الأهداف: هدف هذه الدراسة هو لتقييم قيمة فحص نخاع العظام في تشخيص الأمراض الدموية وتحديد تواتر وأسباب نخاع العظم الجاف.

المرضى والطرق: هذه دراسة استطلاعية فقط وتمت خلال الفترة من يناير 2013 إلى يونيو 2013 في مستشفى هيووا التعليمي هيووا و مستشفى أربيل التعليمي في السليمانية وأربيل على التوالي. وكان عدد إجمالي من 245 حالة خضعت فحص نخاع العظام. وقد استخدم لمس الخزعة في حالة النخاع الجاف. وقد استخدم صبغة السودان في سرطان الدم الحاد.

النتائج: كان عدد المرضى 138 (56.3%) من الذكور و 107 (43.7%) من الإناث، تتراوح أعمارهم 1-81 سنوات، وكان متوسط أعمارهم 36.7 ± 24 عاما. اكثر الحالات شيوعا من المرضى التي خضعت للتقصي السريري وفحص نخاع العظم كانت الشحوب (71.4%)، تليها الحمى (32.2%). وكانت المؤشرات الرئيسية وجود الخلايا السرطانية في الدم 69 (28.2%)، يليها تقييم نخاع العظام لتحديد مرحلة سرطان الغدد الليمفاوية 37 (15.1%). وكانت التشخيصات الشائعة كالاتي: سرطان الدم الحاد 69 (28.2%)، ونخاع نشط (السليبي ليمفوما) 27 (11.1%)، ونخاع نشط 24 (9.8%). وكانت نسبة النخاع الجاف (4.08%) وكانت الأسباب الأكثر شيوعا: سرطان الدم الحاد وتقنية خاطئة (نخاع العادي).

الخلاصة: فحص نخاع العظم هو أداة تشخيصية مهمة في تشخيص وتحديد مراحل مختلفة من الاضطرابات الدموية.

كلمات البحث: نخاع العظم ، خزعة، أمراض الدم، كردستان

Table 1 : Indications for bone marrow examination:

Indication	Frequency	Percentage
Blast cells in peripheral circulation	69	28.2
Bone marrow for lymphoma staging	37	15.2
Anemia	24	9.8
Pancytopenia	20	8.1
Thrombocytopenia	19	7.7
Bicytopenia	15	6.1
Leucocytosis	14	5.6
Polycythemia	14	5.6
Lymphocytosis	13	5.2
Thrombocytosis	6	2.4
Hepatosplenomegaly with anemia	4	1.6
Leucoerythroblastic anemia	2	0.8
To exclude marrow metastasis	8	3.2
Total	245	100

Table 2: Results of Bone marrow examination of the studied cases.

Disorders	Number of cases	%
ALL	40	16.3
AML	29	11.8
Active marrow (negative for lymphoma)	27	11.1
Active marrow	24	9.8
Idiopathic thrombocytopenia purpura	21	8.6
Myeloproliferative neoplasm	21	8.6
Chronic lymphocytic leukemia	13	5.3
Chronic myeloid leukemia	12	4.9
Multiple myeloma	12	4.9
Marrow involvement by lymphoma	10	4.1
Aplastic anemia	10	4.1
Non – diagnostic	6	2.4
Active marrow (negative for solid tumors)	6	2.4
Megaloblastic anemia	5	2
Secondary metastasis	2	0.8
Pure red cell aplasia	2	0.8
Erythroid hyperplasia	2	0.8
Myelodysplastic syndrome	2	0.8
Hairy cells leukemia	1	0.4
Total	245	100

Table 3: Indication of bone marrow examination versus final diagnosis

Indication	Frequency	Final diagnosis	Cases
Acute leukemia	69	ALL AML	40 29
Bone marrow for staging	37	Active marrow (negative for lymphoma) Marrow involved by lymphoma	27 10
Anemia	24	Active marrow Multiple myeloma Erythroid hyperplasia Pure red cells aplasia	13 7 2 2
Pancytopenia	20	Aplastic anemia Megaloblastic anemia Multiple myeloma Non diagnosis marrow Active marrow Hairy cell leukemia	10 4 2 2 1 1
Thrombocytopenia	19	ITP	19
Bicytopenia	15	Active marrow Multiple myeloma Myelodysplastic syndrome ITP CLL Non diagnostic marrow Megaloblastic anemia	5 3 2 2 1 1 1
Leucocytosis	14	CML Non diagnostic marrow Active marrow	12 1 1
Polycythemia	14	Myeloproliferative neoplasm	14
Lymphocytosis	13	CLL Active marrow	12 1
Thrombocytosis	6	Myeloproliferative neoplasm Active marrow	5 1
Hepatosplenomegaly and anemia	4	Active marrow Non diagnostic marrow	2 2
Leucoerythroblastic anemia	2	Myeloproliferative neoplasm	2
To exclude marrow metastasis	8	Active marrow (negative for metastasis) Secondary metastasis	6 2
Total	245		245

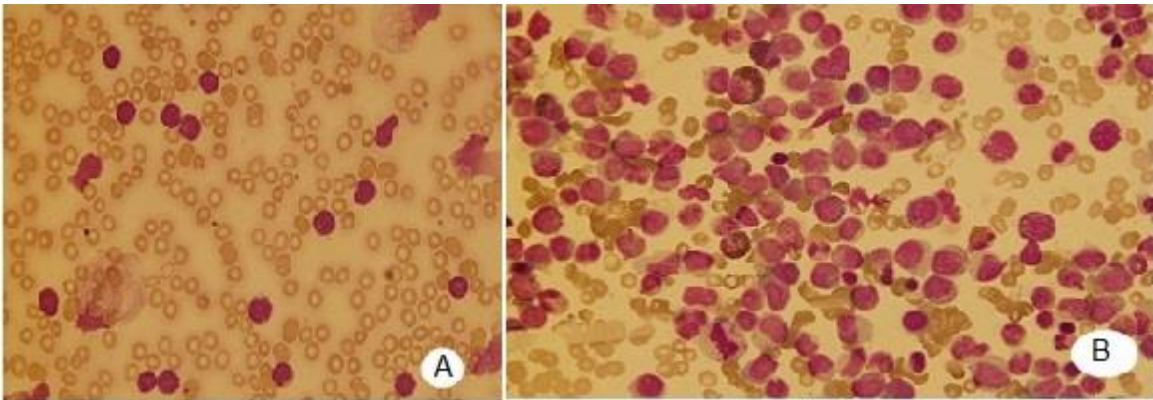


Figure 1:(A) Acute lymphoblastic leukemia.(B) Acute Myeloblastic Leukemia. Marrow aspiration (Leishmann stain)

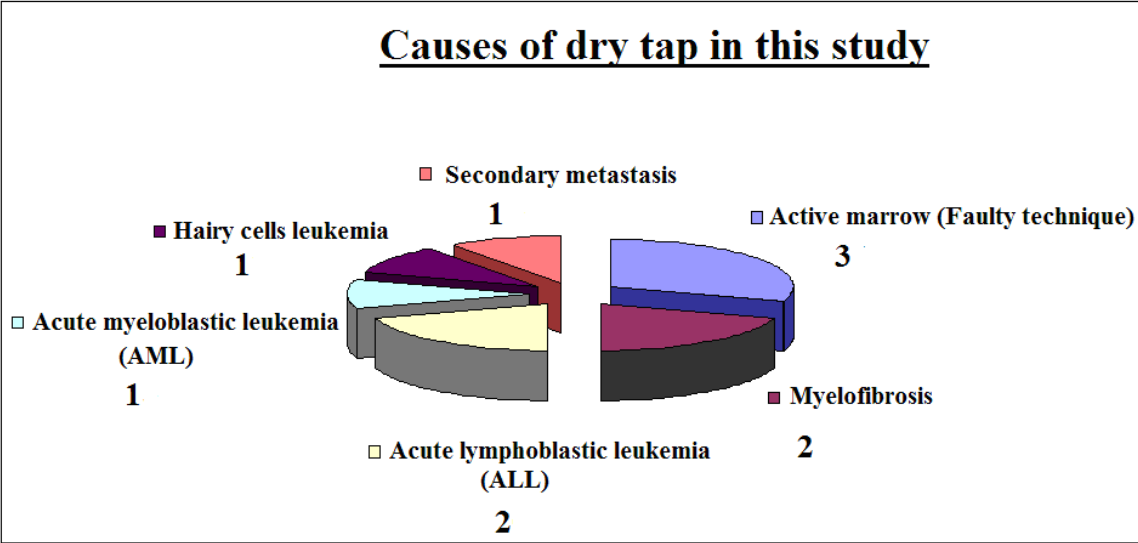


Figure 2 : Causes of dry tap in this study.