

The role of bone marrow aspirate and bone marrow biopsy in the diagnosis of multiple myeloma

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Abstract

Background: Multiple myeloma (MM) is a malignancy of terminally differentiated clonal plasma cells. Expansion of the malignant plasma cells throughout the bone marrow (BM) leads to cytopenias, bone resorption and production of monoclonal immunoglobulins known as M components or paraproteins. BM analysis is an important element in establishing the diagnosis of MM. **The study aims** to evaluate the diagnostic yield of bone marrow aspirate and bone marrow biopsy in patients with MM. and to Compare the newly diagnosed and follow-up cases (regarding the bone marrow examination, hematological and biochemical parameters).

Methods: This is a cross-sectional study conducted from the first of August 2022 to the first of August 2023, and included 32 patients with MM. The patients were diagnosed at the Basrah Oncology and hematology center. The laboratory parameters and BM examination were analyzed and correlated. Immunohistochemical staining with CD138 was done for selected cases.

Results: Anemia (Hemoglobin (Hb) < 10 g/dL) was seen in 59.4% of total cases. Hypercalcemia was found in 9.4% of total cases. Serum creatinine of more than 2 mg/dL was found in 25% of total cases. Monoclonal (M) protein was positive in 84.4% of total cases. There is a significant difference between the positive results of BMA and BMB.

Conclusions: BMA and BMB complement each other for BM evaluation. The combined use of BMA smears, HE stained BMB sections and CD138 IHC is beneficial in obtaining the most clinically relevant BM plasma cell percentage.

Keywords: multiple myeloma (MM), BMA, BMB.

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Introduction

Multiple myeloma (MM) is a mature neoplastic B cell characterized by clonal proliferation of plasma cells in the bone marrow, associated with elevated monoclonal protein in urine and serum resulting in end-organ dysfunction (1).

The precise etiology of MM is unknown. However, frequent alterations and translocations in the promoter genes, particularly chromosome 14, are commonly seen in MM and likely play a role in disease progression (2). The typical features of MM are the destruction of bone (causing bone pain mostly

due to pathological fracture), hypercalcemia, paraprotein in urine and/or blood, immunodeficiency (infection), bone marrow failure (anemia) and excessive immunoglobulin production and deposition (renal failure and amyloidosis). A combination of radiological, laboratory and pathological findings provides the diagnosis of MM(3).

Bone marrow aspirate (BMA) and bone marrow biopsy (BMB) are usually performed to estimate the percentage of abnormal plasma cells ($\geq 10\%$). This percentage is required by the revised International Myeloma Working Group (IMWG) criteria for the diagnosis of MM (2,4).

The IMWG updated the diagnostic criteria for 2022 for MM and both criteria must be met:

1. Clonal bone marrow plasma cells $\geq 10\%$ or biopsy-proven bony or extramedullary plasmacytoma

2. Any one or more of the following myeloma-defining events:

_ Evidence of end-organ damage that can be attributed to the underlying plasma cell proliferative disorder, specifically:

• Hypercalcemia: serum calcium >0.25 mmol/L (>1 mg/dL) higher than the upper limit of normal or >2.75 mmol/L (>11 mg/dL)

• Renal insufficiency: creatinine clearance <40 mL per minute or serum creatinine >177 μ mol/L (>2 mg/dL)

• Anemia: hemoglobin value of >2 g/dL below the lower limit of normal, or a hemoglobin value <10 g/dL

• Bone lesions: one or more osteolytic lesions on skeletal radiography, computed tomography (CT), or positron emission tomography-CT (PET-CT)

_ Clonal bone marrow plasma cell percentage $\geq 60\%$

_ Involved: uninvolved serum free light chain (FLC) ratio ≥ 100 (involved free light chain level must be ≥ 100 mg/L)

_ >1 focal lesion on magnetic resonance imaging (MRI) studies (at least 5 mm in size) (4).

Bone marrow examination is a valuable and economical diagnostic procedure in hematological practice for the diagnosis of both neoplastic and non-neoplastic hematological diseases (5).

Bone marrow aspirate is a perfect tool for studying morphology, differential count and myeloid: erythroid (M: E) ratio. It can be used for flow cytometry and cytogenetics, while BMB gives information regarding cellularity, architecture and focal lesions. BMB not only gives the clue for infiltration but also provides the pattern of involvement and prognostic value (6,7,8).

The appearance of antibody staining has permitted the identification of plasma cells in bone marrow

sections with greater specificity than HE stains, this advance led to more frequent use of bone marrow sections in the diagnosis and follow-up of MM. The use of CD138 (syndecan-1), as an immunohistochemical marker, was a major improvement in the detection of plasma cell infiltrates in formalin-fixed, paraffin-embedded material (9).

Methods

This is an analytical cross-sectional study performed on 32 MM patients, 24 of them were newly diagnosed and 8 cases were on follow-up. All patients were diagnosed at the Basrah Oncology and hematology center/ Al-Sadr Teaching Hospital. The diagnosis of MM was based on the IMWG 2022 diagnostic criteria (4).

All patients were diagnosed by clinical hematologists based on clinical features and laboratory findings including serum protein electrophoresis, bone marrow examination and laboratory investigations for confirmation of the CRAB criteria (Hypercalcemia, renal impairment, anemia and bone lesions).

Newly diagnosed MM cases and cases on follow-up who had simultaneous BMA and BMB were included in the study, while MM cases that did not have BMA and BMB at the same time were excluded.

BMA with a plasma cell percentage of $\geq 10\%$ is regarded as positive, while bone marrow biopsy with plasma cell infiltration ($\geq 10\%$) is regarded as positive.

In this study, the laboratory data (biochemical, hematological and electrophoretic pattern) obtained at the time of diagnosis and during the follow-up period were collected from the patient's medical records at Basrah Oncology and hematology center.

A verbal consent was obtained from the patient himself/herself before enrollment in the study. Also, the study was approved by the Ethical Committee of the Scientific Council of Pathology at the Iraqi Board for Medical Specializations and by the Research Ethics Committee of the Basra Health Directorate.

The standard technique was employed for obtaining the BMA samples using a bone marrow needle from the posterior superior iliac spine. The procedure was done under local anesthesia with 2% injection lidocaine.

For the bone marrow aspirate procedure, the needle 11 G for adults was used. BMB was obtained using a bone marrow trephine biopsy needle, then fixed for a minimum of 24 hours in Bouin's solution. The fixation of the biopsy core was followed by automated tissue processing, paraffin embedding and sectioning (done at the histopathology lab.)

The BMA smears and BMBs were examined for morphological details and reviewed by hematopathologist and histopathologist and the findings of BMAs and BMBs were compared and the final correlation was done.

IHC staining with CD 138 was done for cases that were positive BMA and negative HE-stained BMB sections. The staining was done using a horseradish peroxidase-polymer (two-step detection kit).

Statistical Package for Social Sciences (SPSS) Software (version 27.0 for Windows) was used to perform the statistical analysis for this study.

Results:

The mean age of the cases was 61.38 ± 9.22 years. Fifteen out of 32 cases (46.9%) were in the 7th decade. The youngest patient was male at the age of 45 and the oldest patient was 80 years old female. Twenty-one cases (65.6%) were males and 11 cases (34.4%) were females, with a male: female ratio was 1.9:1. As shown in Table 1.

Table 1 the Age and sex distribution of the participants

Descriptive data	Total cases (N = 32) No. (%)
Sex	
Male	21 (65.6%)
Female	11 (34.4%)
Age groups (years)	No. (%)
40 – 50	6 (18.8%)
51- 60	8 (25%)
61 - 70	15 (46.8%)
>70	3 (9.4%)

Regarding the hematological parameters of the cases,

Table 2 The hematological parameters of participants

Hematological parameters	Newly diagnosed cases (N=24)	Follow-up cases (N=8)	P value
Hb (g/dL) (mean ± SD)	9.8 ± 2.1	9.8 ± 2.3	0.9
WBC ($\times 10^9$ /L) (mean ± SD)	4.6 ± 2.7	5.5 ± 1.7	0.4
Platelets ($\times 10^9$ /L) (mean ± SD)	227.8 ± 109.3	242.5 ± 514.3	0.7
ESR (mm/hr) (mean ± SD)	99 ± 18	81 ± 36	0.07

* Significant at $P < 0.05$ * SD (standard deviation)

Anemia (Hb < 10 mg/dL) was found in 14 newly diagnosed cases (58.3%) and in 5 follow-up cases (62.5%). Low white blood cell (WBC) count $< 4 \times 10^9$ / L was found in 10 newly diagnosed cases (41.7%). None of the follow-up cases showed

leukopenia. Low platelet count was seen in 3 newly diagnosed cases (12.5%), with none of the follow-up cases showing thrombocytopenia. ESR \geq 100 mm/hr was seen in 10 newly diagnosed cases (41.7%) and 2 cases (25%) on follow-up. (figure 1)

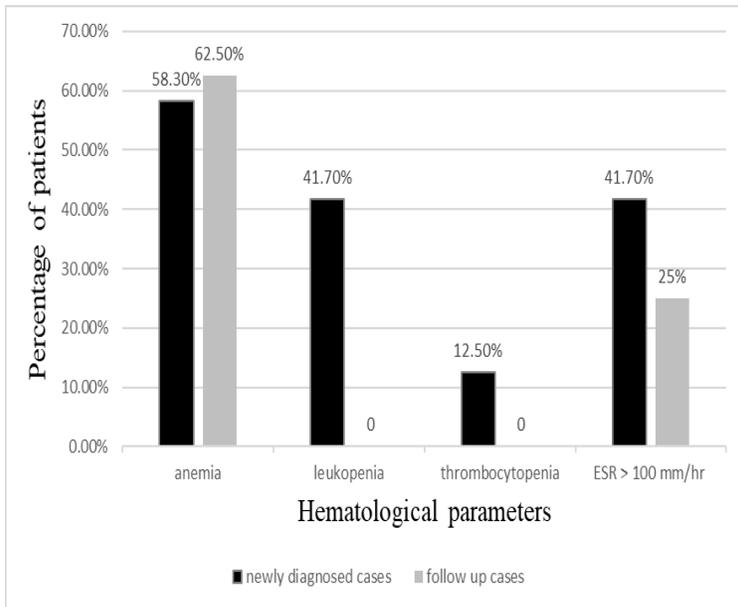


Figure 1 Comparison between the newly diagnosed and follow-up cases regarding the hematological parameters

No significant differences were found between the newly diagnosed cases and the follow-up cases with p-value > 0.05.

The biochemical parameters of the cases were:

Table 3 The biochemical parameters of the participants

Biochemical parameters		Newly diagnosed cases (N = 24)	Follow up cases (N = 8)	P value
Monoclonal protein spike	Positive	22 (91.7%)	5 (62.5%)	0.04
	Negative	2 (8.3%)	3 (37.5%)	
Blood urea (mg/dL) (mean \pm SD)		48.8 \pm 16.5	47.3 \pm 15.6	0.8
Serum creatinine (mg/dL) (mean \pm SD)		1.6 \pm 0.7	1.4 \pm 1.1	0.6
Serum calcium (mg/dL) (mean \pm SD)		9.9 \pm 0.9	10.3 \pm 0.9	0.3

Elevated blood urea was seen in 16 newly diagnosed cases (66.7%) and 6 follow-up cases (75%). Serum creatinine > 2 mg/dL was found in 7 newly diagnosed cases (29.2%) and in only one follow-up case (12.5%). Hypercalcemia (serum calcium > 11 mg/dL) was found in 2 newly diagnosed cases (8.3%), with only one follow-up case (12.5%) showing hypercalcemia. All biochemical parameters showed no significant difference between the newly diagnosed and follow-up cases except monoclonal protein spike which was significantly lower in follow-up cases than the newly diagnosed cases. (figure 2)

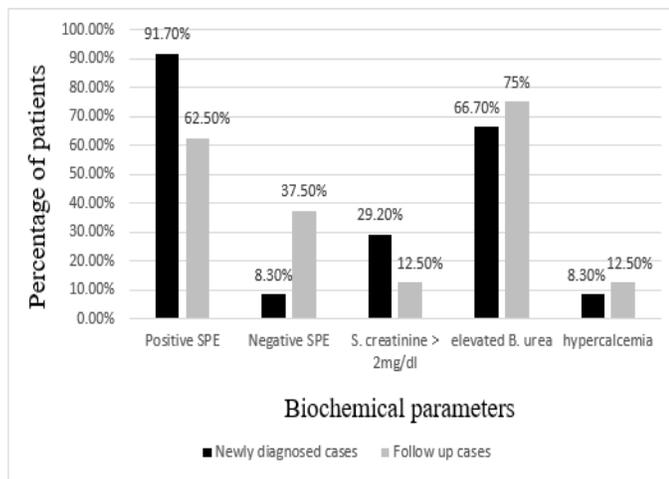


Figure 2 Comparison between the newly diagnosed and follow-up cases regarding the biochemical parameters

Bone marrow findings:

The mean plasma cells percentage was (32.4 ± 18.9) in newly diagnosed patients, and (14.3 ± 15.9) in patients on follow-up, which showed a significant difference between the newly diagnosed and follow-up cases, with a p-value = 0.03. Tables (4 and 5.)

Table 4 BMA findings among participants

BMA	Newly diagnosed cases (N=24)	Follow-up cases (N=8)	P value
Mean plasma cells percentage (%)	32.4 ± 18.9	14.3 ± 15.9	0.03
Typical morphology	4 (17.4%)	5 (62.5%)	
Malignant morphology	19 (82.6%)	3 (37.5%)	

Table 5 BMB findings among participants

BMB (Pattern of infiltration)	Newly diagnosed cases (N=24) (No./%)	Follow-up cases (N=8) (No./%)
Interstitial	9 (37.5%)	2 (25%)
Diffuse	8 (33.3%)	0 (0%)
Mixed	6 (25%)	2 (25%)
No infiltration	1 (4.2%)	4 (50%)

Comparison between BMA and BMB:

Table 6 The Comparison between BMA and BMB (both newly diagnosed and follow-up cases).

	Positive BMB (No./%)	Negative BMB (No./%)	Total (No./%)
Positive BMA (No./%)	24 (75%)	2 (6.2%)	26 (81.2%)
Negative BMA (No./%)	3 (9.4%)	3 (9.4%)	6 (18.8%)
Total (No./%)	27 (84.4%)	5 (15.6%)	32 (100%)

There is a significant difference between the positive results of BMA and BMB with a P value of 0.034.

IHC was done for two cases (6.2%) with positive BMA and negative BMB stained by HE. One of these cases was newly diagnosed and the other case was on follow-up. These two cases were stained immunochemically for CD138 and the results were negative for both cases.

Discussion:

In this study, a comparative evaluation of BMA and BMB was done to determine the diagnostic yield of both procedures, separately and in combination.

In this study, the mean age of the MM patients included was 61.38 ± 9.22 years, with a range of 45 to 80 years. Twelve patients (46.88%) were between 61 and 70 years. These results were comparable with Iraqi studies (10,11), a Saudi Arabian study (12) and an Indian study (3).

The majority of the MM cases in our study were males, with a male-to-female ratio of 1.9:1, which is in agreement with an Iraqi study (13), and Indian studies (3,8). However, these results disagreed with other Iraqi studies (14) and a study was done in Croatia (15).

Regarding the newly diagnosed cases, the mean age was 62.42 ± 9.3 years, with the highest peak in the age group 61-70 years, which is in agreement with an Iraqi study (16), a Libyan study (17), a Saudi Arabian study (18) and an Indian study (3).

In the current study, the mean Hb level in newly diagnosed cases was 9.8 ± 2.1 g/dL, which is comparable with an Iraqi study (16), an Egyptian study (19) and a Libyan study (17). In this study, Hb value of < 10 g/dl was seen in 14 of the newly diagnosed cases (58.3%), which is comparable to what was reported by a Saudi Arabian study (12), a Bangladeshi study (20) and a UK study (21). However, an Indian study (22) reported anemia with Hb < 10 mg/dl in 92.8% of cases. The mean Hb level of follow-up cases were 9.8 ± 2.3 mg/dl, which is not significantly different from the Hb level of the newly diagnosed cases. The mean white blood cell (WBC) count of newly diagnosed cases was $4.62 \pm 2.68 \times 10^9/L$, which is comparable to what was reported by Sultan et al. (23) and by Alwan AF (11). WBC count $< 4 \times 10^9/L$ was seen in 10 patients (41.7%), which is comparable with another study (20). The mean WBC count of follow-up cases was $5.48 \pm 1.72 \times 10^9/L$, which is not significantly different from the newly diagnosed cases.

Regarding the platelet count of newly diagnosed cases, the mean platelet count was 227.8 ± 109.3

$\times 10^9/L$, which is similar to what was reported by two Iraqi studies (11,16). However, thrombocytopenia with a count $< 100 \times 10^9/L$ was observed in 3 cases (12.5%) in this study which is comparable with another study (22), but lower than that reported by other studies (8,20). The mean platelets count of cases on follow-up was $242.5 \pm 514.3 \times 10^9/L$ and there is no significant difference between the newly diagnosed and follow-up cases.

The mean ESR was 99 ± 18 mm/ hr, this is in agreement with a Libyan study by Hussain A (17) and an Iraqi study by Alwan AF (11). ESR ≥ 100 mm/hr was seen in 10 newly diagnosed cases (41.7%), which is lower than that reported by a Bangladeshi study (20). The mean ESR of follow-up cases was 81 ± 36 and there is no significant difference between the two groups.

Regarding the biochemical parameters of the newly diagnosed cases, the monoclonal protein band was detectable in 91.7% of cases on serum protein electrophoresis. Only 2 newly diagnosed cases (8.3%) had normal serum protein electrophoresis, which is comparable with other studies (22,24).

Considering the other biochemical parameters of the newly diagnosed cases, the mean of serum creatinine was 1.6 ± 0.7 mg/dl, which is comparable with what was reported by two Iraqi studies (11,16), serum creatinine levels more than 2mg/dL was found in 29.2% of the newly diagnosed cases indicating the impaired renal function. These results are comparable to a Libyan study (17) and an Indian study (3), while it is lower than what was reported by another Indian study (22).

The mean of blood urea was 48.8 ± 16.5 mg/dl, which is comparable with an Iraqi study by Salih ZM (16), but lower than that reported by other Iraqi studies (18).

The mean of serum calcium was 9.9 ± 0.9 mg/dl, which is comparable to what was reported by a Libyan study (17) and Iraqi studies (11,16). Hypercalcemia was found in 8.3%. These results were comparable to what was reported by a Libyan study (17) but lower than that reported in other studies (11,22).

Regarding bone marrow examination, which is a cornerstone in the diagnosis of MM. Out of the total cases, 26 cases (81.2%) had positive BMAs, while 27 cases (84.4%) had an infiltration by BMB stained with HE. One newly diagnosed case in which the BMA was a dry tap, showed a diffused pattern of plasma cell infiltration in BMB (by myeloma defining events, this case should be regarded as positive regardless of the result of BMA) (4). This could be due to packed marrow which is an important cause of dry tap. However, one newly diagnosed case and one follow-up case had a lower plasma cell percentage than the required level for positivity, this could be due to patchy infiltration of plasma cells or due to sampling error since their BMB were positive for marrow infiltration. These results are correlated well with Indian studies (5,6,8,25).

Regarding the pattern of infiltration in newly diagnosed cases, the majority of the newly diagnosed cases showed interstitial and diffuse patterns of infiltration in 9 cases (37.5%) and 8 cases (33.3%) respectively. Six cases (25%) showed a mixed (focal and interstitial) pattern of infiltration and only a single case showed no infiltration, which is comparable with other studies (8,22). While the follow-up cases showed 25% for each interstitial and mixed pattern and half (50%) of follow-up cases showed no infiltration at all. None of the follow up cases showed a diffuse pattern of infiltration.

The utility of plasma cell quantification by CD138 IHC has been widely supported. It has been generally accepted as a sensitive method for quantifying plasma cell burden, especially in patients with a low percentage of plasma cells on BMA examination (15).

In the present study, an IHC method with CD138 on BMB was used for cases were positive BMA and negative BMB, one of them was a newly diagnosed case and the other was a case on follow-up. The result of IHC was negative for both cases. These results confirm our findings in HE stained BMB and this could be due to patchy infiltration of malignant plasma cells.

In this study, all patients underwent BMA and BMB simultaneously and there is a significant difference

between the positive results of BMA and BMB with a p-value of 0.034.

Considering our results, it can be concluded that BMB is of complementary value for direct estimation of the tumor load in the diagnosis of new and follow-up cases of MM in addition to BMA. These findings agreed with the studies conducted using BMA and trephine biopsy together (26,27).

The combined use of BMA smears HE-stained BMB sections and CD138 IHC is beneficial in obtaining the most clinically relevant bone marrow plasma cell percentage. Further studies with larger numbers of patients are recommended to confirm our results. IHC with CD138 is recommended in a larger number of cases, especially in cases with low plasma cell percentage or negative bone marrow biopsy.

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دور رشفة نخاع العظم و خزعة نخاع العظم في تشخيص الماييلوما المتعددة

الخلاصة

خلفية الدراسة: الماييلوما المتعددة هو ورم خبيث في خلايا البلازما النسيطة المتميزة بشكل نهائي. توسعة الخلايا الخبيثة في جميع أنحاء نخاع العظم يؤدي الى قلة الكريات، وارتشاف العظم وإنتاج الجلوبيولين المناعي وحيد النسيطة المعروف بإسم مكون M

يعد فحص نخاع العظم عنصرا مهما في تشخيص الماييلوما.

الهدف من الدراسة: تهدف الدراسة لتقييم العائد التشخيصي لنضح نخاع العظم وخزعة نخاع العظم في المرضى الذين يعانون من الماييلوما، وللمقارنة بين الحالات المشخصة حديثا والحالات تحت المتابعة (فيما يتعلق بفحص نخاع العظم وقياسات الدم والكيمياء الحيوية).

منهجية الدراسة: هذه دراسة مقطعية أجريت في الفترة من الأول من أغسطس ٢٠٢٢ الى الأول من أغسطس ٢٠٢٣، وشملت ٣٢ مريضا يعانون من الماييلوما المتعددة.

وتم تشخيص المرضى في مركز البصرة للأورام وامراض الدم. تم تحليل المعالم المختبرية وفحص نخاع العظم وإيجاد العلاقة بينها. تم اجراء التلطخ المناعي الكيميائي ب CD138 لحالات مختارة.

النتائج: وجد فقر الدم (الهيموجلوبين اقل من ١٠ جم اديسيلتر) في ٥٩,٤% من اجمالي الحالات. وجد فرط كالسيوم الدم في ٩,٤% من اجمالي الحالات. وجد ان الكرياتينين في الدم اكثر من ٢ ملغم اديسيلتر في ٢٥% من اجمالي الحالات. كان البروتين وحيد النسيطة ايجابيا بنسبة ٨٤,٤% من مجموع الحالات. وقد وجد ان هناك فرق كبير بين النتائج الإيجابية لنضح نخاع العظم وخزعة نخاع العظم.

الاستنتاجات: فحص نضح نخاع العظم وخزعة نخاع العظم يكملان بعضهما البعض لتقييم نخاع العظم.

الاستخدام المشترك لمسحات نضح نخاع العظم، خزعة نخاع العظم المصبوغة بالإيوسين والهيماتوكسيلين والتلطخ المناعي الكيميائي يعد مفيدا في الحصول على النسبة المئوية لخلايا البلازما في نخاع العظم الأكثر صلة سريريا.