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Pathology

Multiple Myeloma: A Morphological Analysis of Bone Marrow Aspirate and Bone Marrow Trephine Biopsies

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Original Research Article

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Abstract: Multiple myeloma (MM) is a clonal plasma cell disorder that accounts for 1% of all malignancies and 15% of hematologic malignancies. The clinical course ranges from relatively indolent forms to frankly aggressive neoplasia. Although bone marrow study is one of the commonly used investigation for diagnosing multiple myeloma it's staging is predominantly based on other laboratory and clinical features as in Salmon and Durie staging and International staging system. To date there is no reliable criterion for assessing the prognosis, based on the bone marrow studies. The current study is to emphasize the role of combined evaluation of bone marrow aspirate and trephine biopsies, not only for making the diagnosis but also for predicting the prognosis.
Keywords: Myeloma, bone marrow aspiration, trephine biopsy, plasma cells.

INTRODUCTION

Multiple myeloma (MM) is a malignant disorder characterized by proliferation of a single clone of plasma cells derived from terminally differentiated B cells with a survival rate ranging from few months to more than 10 years [1-4]. Prognostically significant investigations like serum beta 2 micro globulin and plasma cell labelling index are predominantly done only in higher centres necessitating the need for predicting the prognosis from basic investigations like bone marrow aspiration [BMA] and bone marrow trephine biopsy [BMT] which is commonly performed in all centres.

In addition to estimating the numbers of plasma cells alone in the bone marrow differential count, the commonly missed factors like plasma cell morphology, tumour cell burden and pattern of plasma cell infiltration also needs to be assessed which significantly correlates with the clinical stage of myeloma.

Our study included the evaluation of plasma cell morphology, tumor cell burden and pattern of plasma cell infiltration in bone marrow examination to emphasize the role of combined evaluation of BMA and BMT biopsy in staging and histomorphological grading of plasma cells for predicting the prognosis.

MATERIALS AND METHODS

The medical records, BMA and BMT biopsies of 34 patients with pathologically proven multiple myeloma were retrospectively assessed. Bone marrow aspirate smears were examined under the scanner [4X], low power [10X], high power [40X] and oil immersion objectives [100X] and were considered satisfactory for inclusion in the study only when marrow particles and free cells were observed. A 500 cell count differential was performed in the cellular trails. The cellularity, evaluation of erythroid, myeloid and megakaryocytic series, their pattern of maturation, M: E Ratio and the percentage of plasma cells were assessed. In addition to these routinely evaluated parameters, the plasma cells were subjected to further morphological analysis and the patients were categorized into three groups based on the predominant morphology of plasma cells according to Carter *et al*. [2] classification as follows

- MATURE PLASMA CELLS: Cells which are very similar to normal plasma cells i.e. cells having abundant basophilic cytoplasm, eccentric nuclei and perinuclear hof.
- IMMATURE PLASMA CELLS: Cells with variable amounts of cytoplasm, eccentric nuclei with one or more nucleoli and diffuse chromatin.
- PLASMABLASTS: Cells with a scant cytoplasm and a large nuclei with clear nucleoli.

Bone marrow trephine biopsies with at least 3 marrow spaces below the subcortical space were selected for the study. The trephine biopsies were evaluated for cellularity, hematopoietic elements and plasma cell burden,. In addition to these routinely evaluated parameters pattern of infiltration, morphological grading of plasma cells and histologic staging was also done based on Bartl *et al*. criteria as follows [5].

According to Bartl *et al*. [5], the myeloma cells were classified into 6 types:

- **Marchalko:** Plasma cells akin to normal mature plasma cells with abundant basophilic cytoplasm, eccentric cartwheel nuclei and perinuclear hof. The predominant growth pattern was interstitial pattern.
- **Small cell:** Plasma cells akin to a small lymphocyte [lymphocytoid plasma cells] with narrow rim of basophilic cytoplasm, small round nuclei with dense chromatin. Perinuclear hof was seen in most of the cells with predominant interstitial growth patttern
- **Cleaved:** Plasma cells with a notched, cleaved or convoluted nuclei. A small perinuclear hof was usually present with packed marrow growth pattern.
- **Polymorphous:** The predominant features include cellular pleomorphism, multinucleation and giant plasma cells with no predominant pattern of infiltration.
- **Asynchronous:** This group is characterized by pronounced nucleo – cytoplasmic asynchrony. Plasma cells have an abundant basophilic cytoplasm with pronounced perinuclear hoff, large nuclei, prominent nucleoli with nodular pattern of infiltration
- **Blastic:** Plasmablasts have moderate amount of basophilic cytoplasm, faint perinuclear hoff and large nuclei, prominent nucleoli with complete replacement of bone marrow.

Bartl *et al*. [5] proposed 3 prognostic groups by comparing these histologic subtypes as follows:

- **MM of low grade malignancy:** Marschalko and small cell type
- **MM of intermediate grade malignancy**: Cleaved, polymorphous and asynchronous cell types
- **MM of high grade malignancy**: Plasmablastic cell type

Myeloma cells of well differentiated and intermediate type were grouped together as plasmacytic type and poorly differentiated type of myeloma cells were considered as plasmablastic type [10]

A histologic staging of multiple myeloma was also done based on the volume of plasma cell infiltration as follows:

- Stage I: < 20% plasma cells
- Stage II: 20 – 50 % plasma cells
- Stage III: > 50% plasma cells

RESULTS

The mean age of the patients was 60.6 years [34 – 80 years] with a male to female ratio of 1.8:1. Hypercellular marrow was observed in all patients with relative suppression of erythroid, myeloid and megakaryocytic series. In BMA study, a plasma cell count of more than 50 % was observed in 20 patients [Table: 1]. According to Carter *et al*. [2] the severity of anaemia was directly proportional to volume of plasma cell infiltration in bone marrow, Similarly patients with haemoglobin level below 5 gm % in our study were found to have plasma cell count of more than or equal to 80% in bone marrow aspirate smears [Fig: 1]. Mature plasma cell morphology [Fig: 2] was observed in 20 patients, immature plasma cells [Fig :3] in six and plasmablastic cells [Fig 4] in eight cases.

BMT biopsy revealed hypercellular marrow in 32 patients and normocellular marrow in 2 patients. Complete marrow replacement was observed in 26 patients [Fig: 5] while interstitial pattern of infiltration [Fig: 6] was seen in four and nodular pattern [Fig: 7] in four patients. The residual hematopoietic marrow was generally decreased. Diffuse pattern [Table 2] was predominant in our study in contrast to the interstitial pattern observed in other studies Bartl *et al*. [5].

Histologic staging [Table 3] of multiple myeloma based on the tumour cell burden was done as per the criteria of [5] Stage III disease was seen in majority of patients [76.4%] at the time of diagnosis. Plasma cell quantification showed more than 50% plasma cell infiltrate in trephine biopsy in 26 patients [76.4%] compared to the same extent of infiltration seen only in 20 patients [58.6%] in bone marrow aspirate indicating the underestimation of plasma cell burden in bone marrow aspirate.

The most commonly observed plasma cell type was the Marschalko type [Fig: 8, Table 4] which were akin to normal plasma cells. Small cell type [Fig: 9] was visualised in 4 patients and blastic type [Fig: 10] in 8 patients. Cleaved cell, polymorphous type and asynchronous morphology were not visualized in any patient. Recognition of these morphological types of plasma cells is necessary to avoid erroneous diagnosis.

Plasma cell morphology in BMT biopsy revealed plasmacytic picture in 26 patients and plasmablastic feature in the remaining 8 patients [Table 5]. Plasma cell infiltration of more than 50% was observed in all patients with plasmablastic morphology. Histologically unfavourable features like plasmablastic morphology, > 50% infiltration and a diffuse pattern of infiltration were observed in 6 cases. A diffuse pattern

of infiltration was observed in similar frequency in patients with plasmablastic [6/8 cases] and plasmacytic morphology [20/26 cases].

Presence of fibrosis in the bone marrow which represents a poor prognostic sign was not observed in any of our cases. Fibrosis cannot be demonstrated in an aspirate study which further indicates the need for evaluation of a trephine biopsy as well.

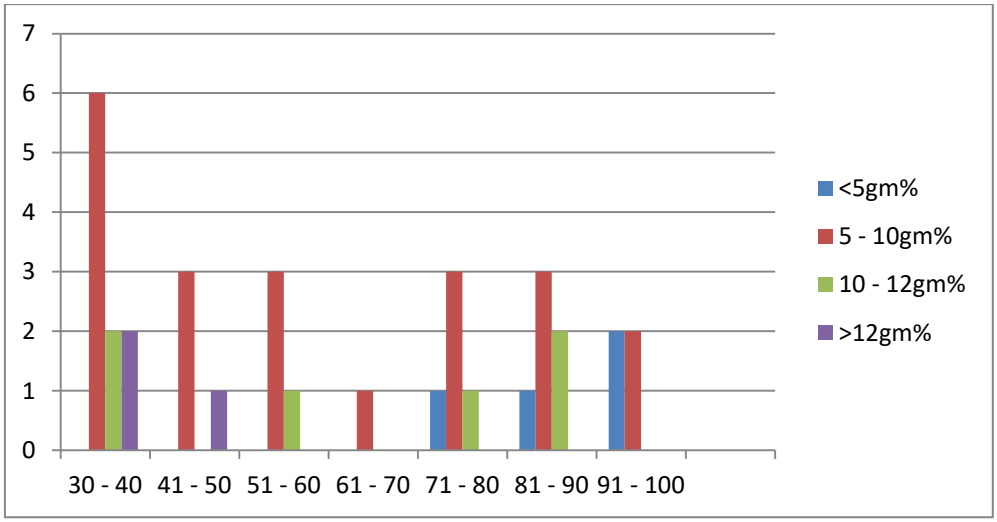

Fig-1: Summarizes the relationship between plasma cell volume in BMA and hemoglobin levels

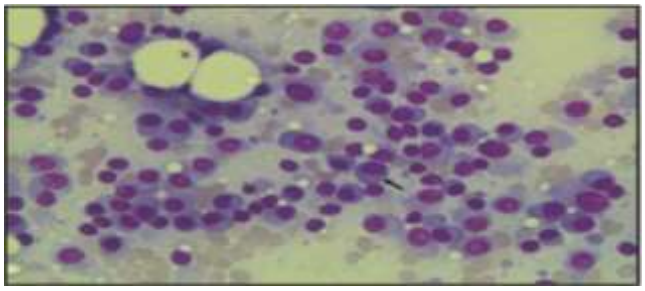

Fig-2: Bone marrow aspirate smears with mature plasma cells. Giemsa stain [40 x]

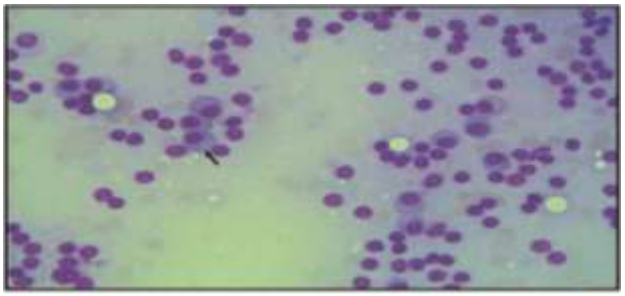

Fig-3: Bone marrow aspirate smears with immature plasma cells. Giemsa stain [40 x]

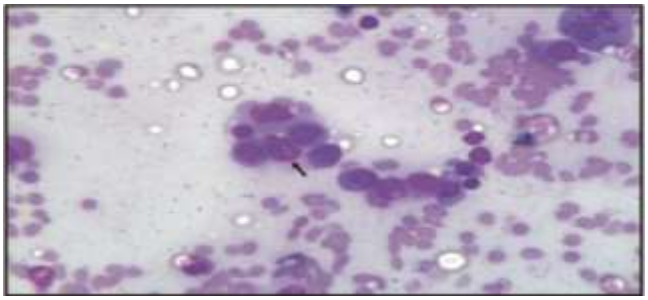

Fig-4: Bone marrow aspirate smears with plasmablastic cells. Giemsa stain [40 x]

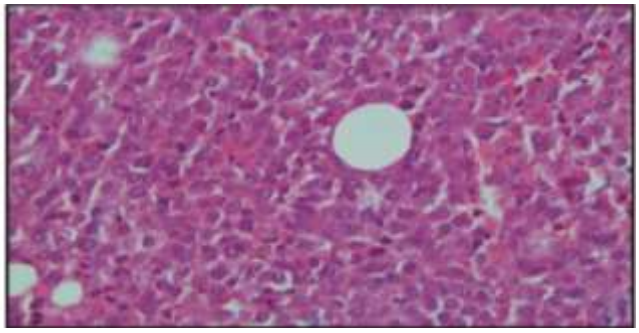

Fig-5: Diffuse pattern of infiltration. H & E stain [40x]

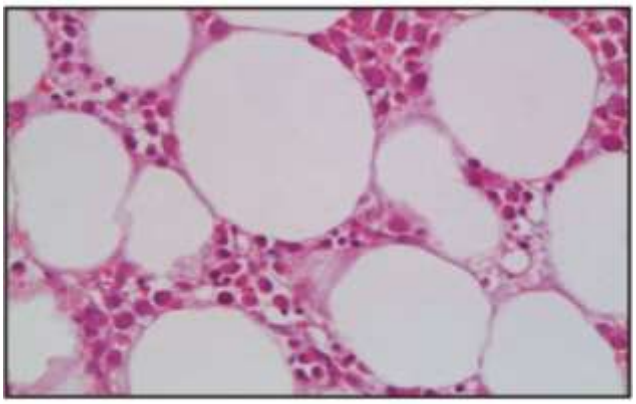

Fig-6: Interstitial pattern of infiltration. H & E stain [40x]

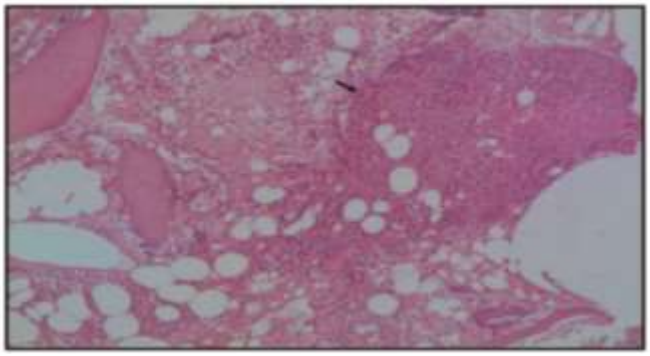

Fig-7: Nodular pattern of infiltration. H & E stain [40x]

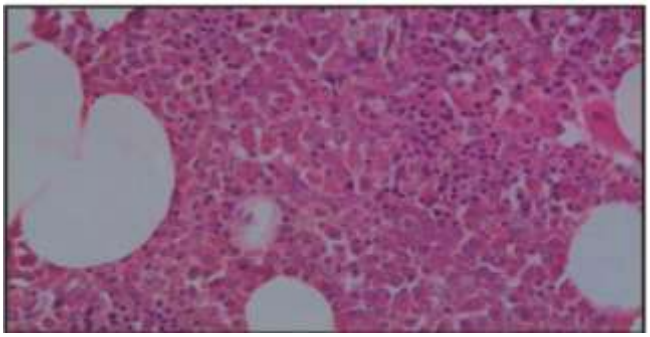

Fig-8: Plasma cells of Marschalko type. H & E stain. [40x]

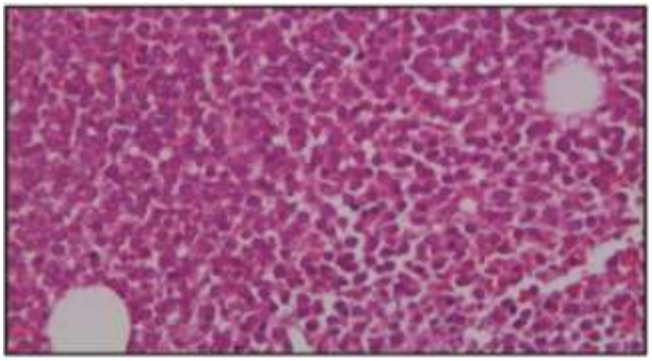

Fig-9: Plasma cells of small cell type. H & E stain. [40x]

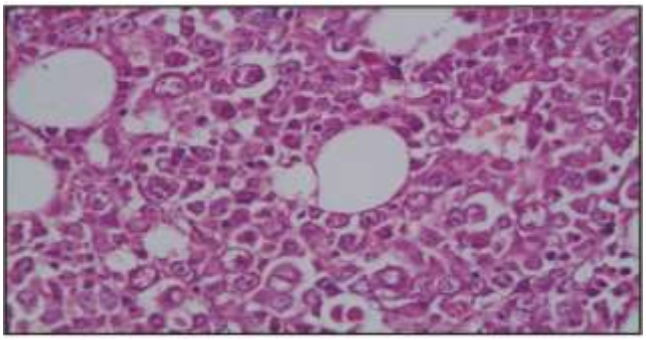

Fig 10: Plasma cells of plasmablastic type. H & E stain. [40x]

Table-1: Percentage Of Plasma Cell Infiltrate In The Aspirate Smears

% Of Plasma Cells	No. Of Patients	Frequency (%)
30 - 40	10	29.4%
41 - 50	4	11.7%
51 - 60	4	11.7%
61 - 70	1	2.9%
71 - 80	5	14.7%
81 - 90	6	17.6 %
91 - 100	4	11.7%
Total	34	

Table-2: Pattern Of Infiltration Of Plasma Cells In Trephine Biopsies

Pattern	No. Of Patients
Diffuse	26
Interstitial	4
Nodular	4
Mixed	0
TOTAL	34

Table-3: Histologic Staging Of Multiple Myeloma

Stage	Plasma Cell Burden	Frequency
I	< 20 %	[0]0%
II	20 – 50 %	[8] 23.5%
III	>50 %	[26] 76.4%

Table-4: Histological Grading Of Plasma Cells

Morphological Subtype	No.Of Patients	Frequency	Histological Grade
MARSCHALKO	22	64.7	LOW
SMALL CELL	4	11.7	LOW
CLEAVED TYPE	0	0	INT
POLYMORPHOUS	0	0	INT
ASYNCHRONOUS	0	0	INT
BLASTIC	8	23.5	HIGH

Table-5: Comparison Of Trephine Biopsy Finding With Plasma Cell Morphology

Parameters		Plasmacytic	Plasmablastic	Total
Volume Of Infiltration	< 20%	0	0	34
	20 – 50 %	8	0	
	>50%	18	8	
Pattern Of Infiltration	INTERSTITIAL	2	2	34
	NODULAR	4	0	
	DIFFUSE	20	6	
Fibrosis	PRESENT	0	0	

DISCUSSION

Multiple myeloma is a clonal plasma cell disorder that has a varied clinical presentation ranging from indolent forms to frankly aggressive neoplasia [2]. The current study presents an approach to analyze the bone marrow aspirate findings in multiple myeloma and to determine the value of trephine biopsy in establishing the accurate diagnosis.

Anemia was observed in 91.1% of our patients which could be due to erythropoietin deficiency resulting from renal failure or direct replacement of marrow by plasma cells [3]. This contrasts with the studies by Bartl *et al*. [5] and Singhal *et al*. [4] in which anemia were found only in lower percentage of patients 68% and 30.6% respectively).

Salmon and Durie staging system categorised patients with myeloma into three groups based on haemoglobin, serum calcium, M component production rate and radiological findings. They were further sub classified based on renal function. These Clinical and laboratory parameters have a well established role in prognosticating multiple myeloma. On the other hand, many authors have demonstrated the relation between plasma cell morphology and prognosis in patients with multiple myeloma. Plasma cells with predominantly plasmablastic feratures were considered to be an unfavourable prognostic group [6,7]. Kuriakose *et al*. and Tsuchiya *et al*. [7, 8] demonstrated significant relationship between plasma cell morphology and duration of survival of the patients emphasizing the importance of including the plasma cell morphology as a prognostic determinant in the already existing system. Such a reproducible form of prognostic classification system is very essential since it forms the basis for selecting the best treatment [9].

Histologic classification systems are widely in use for lymphomas. However no such systems exist for multiple myeloma even though they are derived from terminally differentiated B cells. Subramanian *et al*. [10] analysed the association between the morphology of plasma cells in marrow biopsy and its prognosis. Histologic grading as proposed by Bartl *et al*. [5] was done in our series since it was characterized by a high prognostic relevance. Most cases of multiple myeloma are composed of easily recognisable plasma cells and therefore can be diagnosed without any difficulty [11]. However there are a considerable proportion of tumours which pose a diagnostic problem since they exhibit unusual cytologic features. Failure to recognize such variants can result in erroneous or under diagnosis. As histopathologists are generally unaware of such unusual forms of plasma cells, histologic grading of plasma cells is mandatory to overcome this problem.

Although bone marrow aspiration is preferable for studying the morphology of plasma cells, the plasma cell burden is often under estimated in it necessitating the need for trephine biopsies. Similar observation was seen in the study by Terpstra *et al*. [12] such disparity could be due to focal growth pattern or fibrosis. Hence the assessment of plasma cell infiltration based on BMA alone may be inaccurate. Moreover, patient with early stages of multiple myeloma respond well to chemotherapy and have an excellent prognosis. A bone marrow biopsy is often indicated in these patients for the diagnosis of minimal infiltration as seen in 23.5 % of our patients. Bone marrow fibrosis which is a poor prognostic sign cannot be demonstrated in aspirate study further emphasizing the need for performing additional trephine biopsy.

While an interstitial pattern was the predominant pattern of infiltration in studies by Bartl *et al*. [5], most cases of myeloma showed a diffuse pattern

in our study. According to Stifter *et al*. [12] the pattern of infiltration was proportionate to the stage of disease with preservation of hematopoiesis in interstitial and nodular patterns in contrast to diffuse pattern which showed suppression of hematopoiesis. In our study, even though diffuse pattern of infiltration was seen in 26 biopsies the residual hematopoiesis was suppressed in 32 cases.

The following factors can be better assessed in a trephine biopsy:

- Volume of plasma cell infiltration
- Pattern of plasma cell infiltration
- Histologic grading
- Grading of fibrosis &
- Plasma cell quantification by IHC

Hence, in any case of suspected myeloma a bone marrow trephine biopsy must be performed in addition to bone marrow aspiration procedure so that any disadvantage that is encountered in one procedure can be overcome by the other.

The findings in our study emphasize combined evaluation of bone marrow aspiration and trephine biopsy and also implicate the need for morphological evaluation of plasma cells in bone marrow aspiration, histologic grading of plasma cells in bone marrow trephine biopsy and quantitaton of plasma cells to supplement the already existing clinical staging systems.

CONCLUSION

In every suspected case of multiple myeloma a combined evaluation of bone marrow aspirate and bone marrow trephine biopsy is preferable in confirming the diagnosis, staging the disease and predicting prognosis. Any limitation in either of these procedures can be overcome by combined evaluation of both the procedures. Prognostically significant parameters like histomorphological grading, histological staging and pattern of infiltration of plasma cells should also be included in the final report in addition to routinely evaluated parameters. However a larger study is advocated to design a scoring system for assessing the prognosis of multiple myeloma.

REFERENCES

1. Larrea D, Fernández C, Rosiñol L, Cibeira MT, Rozman M, Rovira M, Bladé J. Extensive soft-tissue involvement by plasmablastic myeloma arising from displaced humeral fractures. European journal of haematology. 2010 Nov 1;85(5):448-51.
2. Carter A, Hocherman I, Linn S, Cohen Y, Tatarsky I. Prognostic significance of plasma cell morphology in multiple myeloma. Cancer. 1987 Sep 1;60(5):1060-5.
3. Kyle RA, Gertz MA, Witzig TE, Lust JA, Lacy MQ, Dispenzieri A, Fonseca R, Rajkumar SV, Offord JR, Larson DR, Plevak ME. Review of 1027 patients with newly diagnosed multiple myeloma. InMayo Clinic Proceedings 2003 Jan 1 (Vol. 78, No. 1, pp. 21-33). Elsevier.
4. Singhal N, Singh T, Singh ZN, Shome DK, Gaiha M. Histomorphology of multiple myeloma on bone marrow biopsy. Indian journal of pathology & microbiology. 2004 Jul;47(3):359-63.
5. Bartl R, Frisch B, Fateh-Moghadam A, Kettner G, Jaeger K, Sommerfeld W. Histologic classification and staging of multiple myeloma: a retrospective and prospective study of 674 cases. American Journal of Clinical Pathology. 1987 Mar 1;87(3):342-55.
6. Greipp PR, Raymond NM, Kyle RA, O'Fallon WM. Multiple myeloma: significance of plasmablastic subtype in morphological classification. Blood. 1985 Feb 1;65(2):305-10.
7. Kuriakose P, Das S, Mani A. Bone marrow morphology in multiple myeloma. Indian journal of cancer. 1995 Sep;32(3):100-3.
8. Tsuchiya J, Murakami H, Kanoh T, Kosaka M, Sezaki T, Mikuni C, Kawato M, Takagi T, Togawa A, Isobe T, Suzuki K. Ten-year survival and prognostic factors in multiple myeloma. British journal of haematology. 1994 Aug 1;87(4):832-4.
9. Turesson I, Abildgaard N, Ahlgren T, Dahl IM, Holmberg E, Hjorth M, Nielsen JL, Odén A, Seidel C, Waage A, Westin J. Prognostic evaluation in multiple myeloma: an analysis of the impact of new prognostic factors. British journal of haematology. 1999 Sep 1;106:1005-12.
10. Subramanian R, Basu D, Dutta TK. Prognostic significance of bone marrow histology in multiple myeloma. Indian journal of cancer. 2009 Jan 1;46(1):40.
11. Banerjee SS, Verma S, Shanks JH. Morphological variants of plasma cell tumours. Histopathology. 2004 Jan 1;44(1):2-8.
12. Štifter S, Babarović E, Valković T, Seili-Bekafigo I, Štemberger C, Načinović A, Lučin K, Jonjić N. Combined evaluation of bone marrow aspirate and biopsy is superior in the prognosis of multiple myeloma. Diagnostic pathology. 2010 Dec;5(1):30.