Original Research Article

Comparison of bone marrow aspiration cytology, touch imprint cytology and bone marrow biopsy for bone marrow evaluation

Kuntal Patel[1*], Nisarg Savjiani[1], Bhumika Gharia[1], J.J.J. Falleiro[2]

[1]MD (Pathology), SBKS Medical Institute and Research Centre, Vadodara, Gujarat, India
[2]Professor, Pathology Department, SBKS Medical Institute and Research Centre, Vadodara, Gujarat, India
[*]Corresponding author email: **kuntal2789@gmail.com**

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Abstract

Introduction: For diagnosis of hematological disorders there are three modalities to examine bone marrow, bone marrow aspiration cytology (BMA), bone marrow biopsy (BMB) and touch imprint cytology (BMI). BMA gives cytological picture, BMI also gives cytological picture but cells are less in number and BMB gives cytological as well as bone marrow architectural picture. BMA alone may not be sufficient to reach diagnosis therefore the present study was undertaken to compare the above three modalities.

Material and methods: The present study was a prospective study done from January 2013 to December 2013. Total 51 cases, where BMA, BMI and BMB were performed on OPD and IPD patients at Dhiraj General Hospital, Vadodara were included. Complete clinical data were recorded including physical examination, complete hematological study along with other relevant investigations and proforma filled.

Results: The various diseases diagnosed by BMA, BMI and BMB were megaloblastic anemia (19.6%), aplastic/ hypoplastic anemia (13.7%), iron deficiency anemia/ micronormoblastic erythroid hyperplasia (2.0%), dimorphic anemia (5.9%), idiopathic thrombocytopenic purpura (2.0%), plasma cell dyscrasias (3.9%), Myeloproliferative disorders (3.9%), leukemia (15.7%), normocellular marrow (13.7%), metastasis (15.7%) and miscellaneous (3.9%).

Conclusion: BMA is found to be the superior procedure for evaluation of hematological disorders compared to BMI and BMB.

Key words

Bone marrow aspiration, Bone marrow imprint, Bone marrow biopsy.

Introduction

The bone marrow can be examined by Bone Marrow Aspiration cytology (BMA), Bone Marrow Biopsy (BMB) and Bone Marrow Imprint (Touch Imprint) cytology (BMI). BMA gives cytological picture, BMI gives cytological picture but cells are less in number and BMB gives cytological picture as well as bone marrow architecture. The BMA alone may not be sufficient to make a diagnosis. The present study was undertaken to compare the above three modalities for bone marrow evaluation. The technique of BMA is universally accepted and widely used. However, BMB, as a diagnostic procedure, is being increasingly used for the study of hematological pathology and could possibly be the only way for reaching a correct diagnosis. If performed correctly, BMA is simple and safe and can be repeated many times and even performed on outpatients. It seems to be safe in almost all circumstances, even when thrombocytopenic purpura is present [1]. However, when there is a major disorder of coagulation, such as hemophilia, BMA should never be attempted without appropriate cover and checking by Coagulation Factor Assay prior to the procedure. BMB is little less simple, but can also be performed on outpatients. BMI is also a reliable diagnostic tool for determining the cellular composition. The bone marrow evaluation may either confirm clinically suspected disease or provide previously unsuspected diagnosis. Although studies have evaluated the role of BMA in diagnosing various hematological disorders but fewer studies have compared the relative value of BMB and BMI. The present study comprises of 51 BMA, BMB and BMI carried out at Dhiraj General Hospital, Vadodara, to compare relative amount of information obtained in each procedure.

Material and methods

The present study was prospective study consisted of 51 patients of inpatient and outpatient departments of Dhiraj General Hospital, Vadodara, where BMA, BMI and BMB were performed from January 2013 to December 2013. Complete clinical data were recorded including physical examination, complete hematological study along with other relevant investigations and pro-forma filled.

Inclusion criteria

Indications for bone marrow examination with due informed consent of patients admitted to or attending OPD in Dhiraj General Hospital [2].

Indications for BMA

- Red cells disorders (e.g. Pancytopenia, Pure red cell aplasia)
- Leukocytic disorders (e.g. Subleukemia and aleukemic leukemia, acute leukemia)
- Megakaryocytic and platelets disorders (e.g. Unexplained thrombocytopenia and thrombocytosis)
- Myeloproliferative disorders
- Myelodysplastic syndromes
- Paraproteininemias
- Pyrexia of unknown origin
- Suspected lysosomal or other storage disorders
- Iron store assessment
- Metastasis
- Unexplained hepatomegaly and/or splenomegaly

To correlate information obtained on BMA with BMI and BMB and to evaluate the necessity for BMB and BMI, bone marrow BMA and bone marrow BMB were performed in all cases.

Procedure for examination [3, 4]

BMA, BMI and BMB were evaluated after taking detailed clinical history. PBSs were taken at the time of BMA/BMB, stained with Romanowsky stain and evaluated followed by BMA/BMI examination.

BMA/ BMI smears

- Smears were chosen having marrow particles and cell trails of particles.
- Under low power cellularity, megakaryocytes and presence of metastatic carcinoma cells if any were assessed.
- Area was selected in the cell trail of the particle where dilution was not present and cell morphology were best made out and differential count was carried out on at least 500 nucleated cells other than erythroid precursors using oil immersion.
- M: E ratio was then calculated.
- Perl's stained BMA were examined under lower power (10X) to assess storage iron. Examination under high power (40X) and oil immersion (100X) was done to assess whether siderotic granulation was reduced, normal or increased in normoblasts and macrophages, and to detect abnormally prominent iron deposits.

BMB

- Under low power, general impression of the biopsy, including overall cellularity, architecture and megakaryocyte number and distribution, abnormalities of the bone, focal lesions, such as granulomas or infiltrates of metastatic tumor or lymphoma were studied. Topographical relationship between bony trabeculae and the marrow was assessed.
- Following this, the bone, hemopoietic elements and marrow stromal elements were studied using medium power (10x objective) and a high power(40X objective)
- Examination under oil immersion (100X objective) was not done.

Comparative evaluation was based on

- Ease of technique used
- Adequacy of materials obtained
- Cytomorphology, cellularity and architecture
- Utility in diagnosis of different disorders

Results

Based on the hematological findings and other relevant investigations, the cases were broadly classified into 11 groups and were diagnosed based on BMA, BMI and BMB were as per **Table - 1**. The positive correlation between these three diagnostic tools was as per in **Table - 2**. BMI alone was not diagnostic in any of the case. But, it was found to be supplementary in following cases. (**Table - 3, 4**)

Table - 1: 51 cases diagnosed based on BMA, BMI and BMB.

Sr. No.	Diagnosis (Total no. of cases)	BMA	BMI	BMB
1	Megaloblastic anemia (10)	10	8	9
2	Aplastic/ hypoplastic anemia (7)			7
3	Microcytic hypochromic anemia (1)	1		
4	Dimorphic anemia (3)	3	1	1
5	Idiopathic thrombocytopenic purpura (1)	1	1	1
6	Plasma cell dyscrasias (2)	2	2	2
7	Myeloproliferative disorder (2)	1		2
8	Acute leukemia (8)	7	2	2
9	Normocellular marrow (8)	8	6	6
10	Metastasis (7)	6	2	6
11	Miscellaneous (2)	2	1	1
	Total (51)	41	23	37
	Percentage (%)	80.39	45.10	72.55

Table - 2: Positive correlation between BMA, BMI and BMB.

Sr. No.	Diagnosis (Total)	BMA and BMI	BMA and BMB	BMB and BMI
1	Megaloblastic anemia (10)	8	9	8
2	Aplastic/ hypoplastic anemia (7)	7	7	7
3	Microcytic hypochromic anemia (1)	0	0	0
4	Dimorphic anemia (3)	1	1	2
5	Idiopathic thrombocytopenic purpura (1)	1	1	1
6	Plasma cell dyscrasias (2)	2	2	2
7	Myeloproliferative disorder (2)	1	2	1
8	Acute leukemia (8)	3	3	5
9	Normocellular marrow (8)	6	6	8
10	Metastasis (7)	6	3	4
11	Miscellaneous (2)	1	1	2
	Total (51)	36	35	40
	Percentage (%)	70.59	68.62	78.43

Table - 3: Cases diagnosed by BMA where others were supplementary.

Sr. No.	Diagnosis (Total diagnosed by BMA)	BMB	BMI
1	Megaloblastic anemia (10)	9	8
2	Microcytic hypochromic anemia (1)	0	0
3	Dimorphic anemia (3)	1	1
4	Idiopathic thrombocytopenic purpura (1)	1	1
5	Plasma cell dyscrasias (2)	2	2
6	Myeloproliferative disorder (1)	2	1
7	Acute leukemia (7)	3	3
8	Normocellular marrow (8)	6	6
9	Metastasis (1)	3	6
10	Miscellaneous (1)	1	1
	Total (35)	28	29
	Percentage (%)	80	82

Table - 4: Cases diagnosed by BMB where others were supplementary.

Sr. No.	Diagnosis (total diagnosed by BMB)	BMA	BMI
1	Aplastic/ hypoplastic anemia (7)	0	0
2	Myeloproliferative disorder (1)	1	1
3	Acute leukemia (1)	0	0
4	Metastasis (6)	3	4
5	Miscellaneous (1)	1	1
	Total (16)	5	6
	Percentage (%)	31.25	37.5

Discussion

The comparative evaluation of BMA, BMI and BMB was undertaken to assess whether all three procedures are required in every case or in certain cases only BMA or only BMB would give adequate diagnostic information, thereby relieving the patient from unnecessary additional stress. In thirty one cases all three procedures were performed. In rest of the cases only one or two procedures were done.

On comparison with the study by Nanda A., et al. [5], the p value is found to be significant. Nanda A., et al. [5], in their study has found that BMA alone was sufficient in making a diagnosis in 88.6% cases. In the remaining 11.4% cases, BMB was necessary for making a diagnosis due to incomplete information provided by BMA or its inability to give a correct diagnosis. These cases were mostly hypoplastic, aplastic marrow, myelofibrosis, and marrow infiltration by metastatic tumours and lymphomatous infiltrations. (**Table – 5**)

Table - 5: Comparison of diagnostic accuracy of BMA and trephine BMB.

Study	**BMA (%)**	**BMB (%)**
Nanda A., et al. (2002) [5]	88.6	11.4
Pandya A., et al. (2012)	70	30
Smita Chandra, et al. (2011)	70	30
Present study	68.62	31.38

In the present study of 51 cases, BMA was diagnostic in 68.62% cases and trephine BMB was diagnostic in 31.38% cases where BMA mainly was a dry tap or diluted with blood. BMI was supplementary to BMI in 82% and supplementary to BMB in 37.5 % of cases. It was found that complete clinical and other relevant parameters (i.e. laboratory and radiological findings) were needed in evaluating the BMA, BMI and BMB to arrive at a conclusive diagnosis by ruling out other differential diagnosis.

In all cases, the cytomorphology was the best in BMA (**Figure - 1**, **2**, **3, 4**) followed by BMI (**Figure - 5, 6, 7**) and least in BMB (**Figure - 8**, **9**, **10**, **11**) since the BMA/BMI had not been anti-coagulated or stored before making smears. BMI were made by either gentle rolling or touching trephine specimen, since they were not spread smears. Therefore cells overlapped with each other. While in BMB tissue processing the cells got shrunken. Comparative values, merits and demerits of different techniques were explained as per **Table – 6**.

Figure – 1: Microphotograph of BMA showing hyper cellular marrow (4X) in MA. Inset showing megaloblasts.

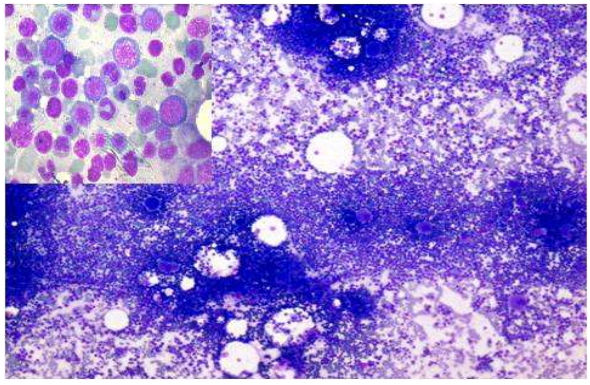


Figure – 2: Microphotograph of BMA showing Hypocellular Marrow in AA. (10X)

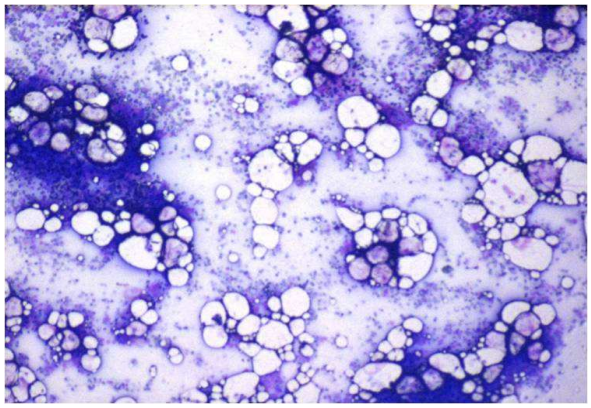


Figure – 3: Microphotograph of BMA showing PCs in Plasma Cell Dyscrasia. Tri-nucleated Plasma Cell is best appreciated (60X)

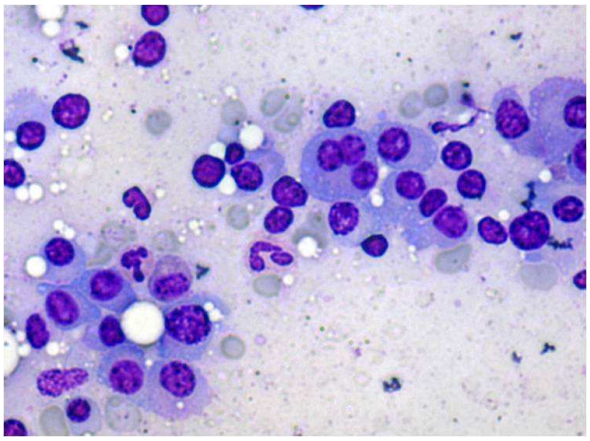


Figure – 4: Microphotograph of BMA showing intracellular LD Bodies in kalaazar. (60X)

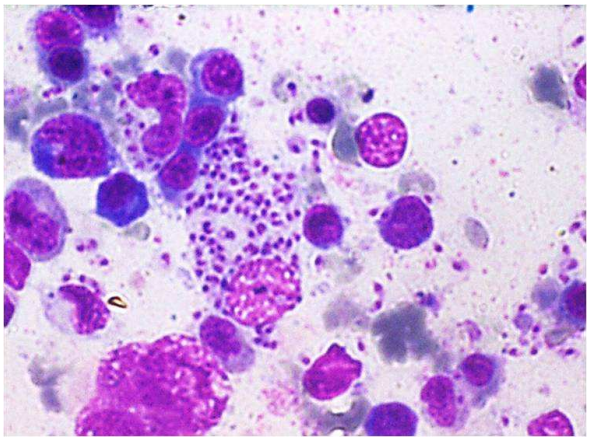


Figure – 5: Microphotograph of BMI showing hyper-cellularity in MA. Morphological identification is difficult than BMA due to overlapping of cells. (10X)

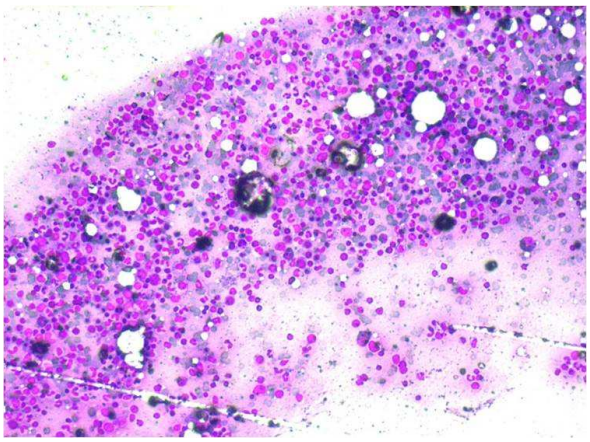


Figure – 6: Microphotograph of BMI showing hypo-cellularity in AA. (10X)

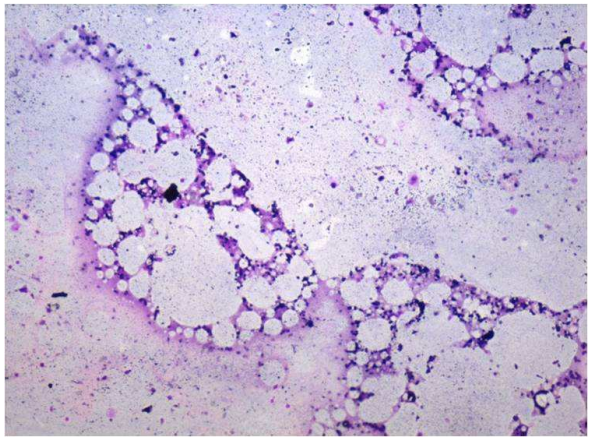


Figure – 7: Microphotograph of BMI in Plasma Cell Dyscrasia. Cell crowding affects morphological identification of cells. (20X)

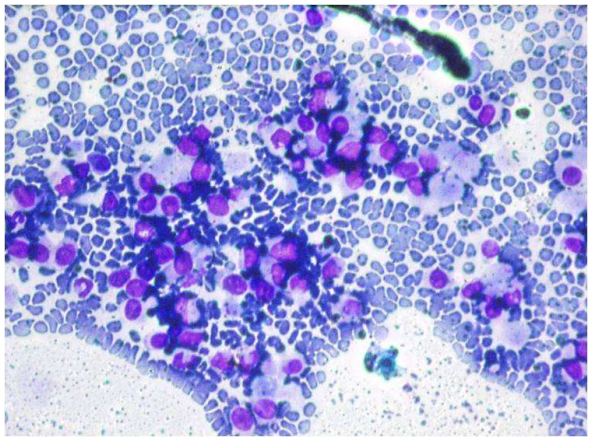


Figure – 8: Microphotograph of BMB showing hypocellular marrow with arrow showing island of marrow cells in AA. BMB is so the diagnostic in case of AA (20X).

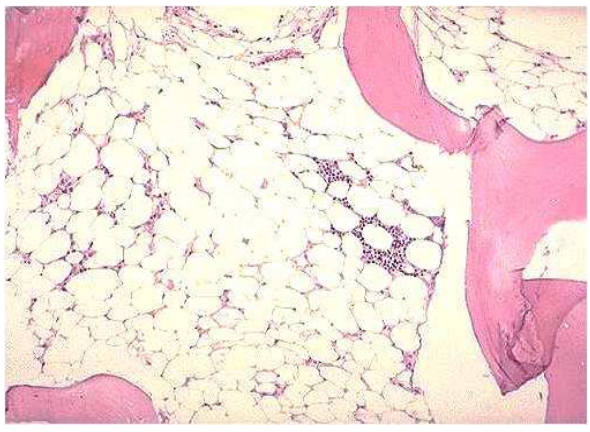


Figure – 9: Microphotograph of BMB showing fibrosis and streaming of cells. In case of MPN – IMF, BMB is the diagnostic aid. (10X)

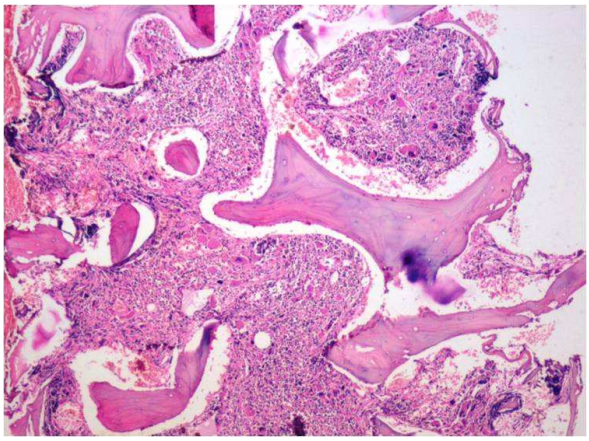


Figure – 10: Microphotograph of shows BMB diagnosed as ALL. BMA was dry tap due to packed marrow. (20X)

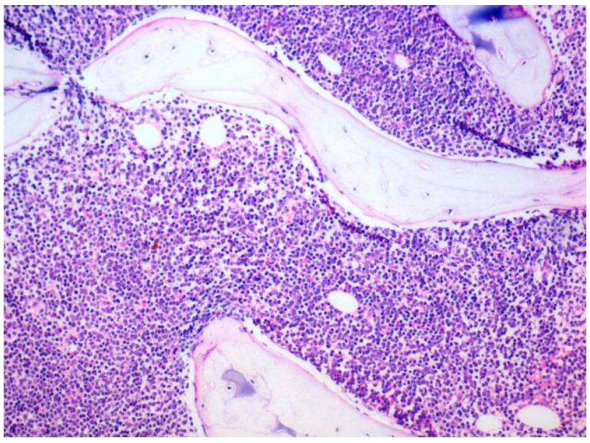
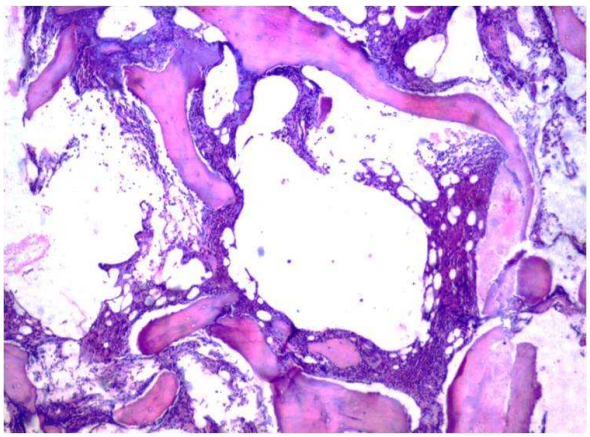


Figure – 11: Microphotograph of BMB showing Paratrabecular pattern in Folicular Lymphoma. (10X)



Conclusion

Bone marrow examination is a safe, quick and easy procedure with very less patient discomfort. It is cost-effective and does not require sophisticated equipments. It may be difficult to perform BMB under local anesthesia in non-co-operative cases and relatively takes longer time to perform with a bit of patient discomfort. BMA shows better cellular details when compared to BMI and BMB. BMB is the diagnostic investigation in dry tap cases like AA, IMF, MDS and metastatic tumors. The cellular architecture is well preserved compared to BMA. The advantages in correct diagnosis of a case by BMA in conjunction with the clinical, hematological, BMI and BMB study, far outweighs the minor disadvantages with BMB. BMA is found to be the superior procedure for evaluation of hematological disorders compared to BMI and BMB.

References

1. Henry's Clinical Diagnosis and Management by Laboratory Methods, 22nd edition, Saunders, 2011.
2. Tejinder Singh. Atlas and Text of Hematology, Fourth edition. Avichal Publishers, 2014.
3. Bain B.J., Bone marrow biopsy. J Clin Pathol., 2001; 54(10): 737-742.
4. Bain B.J. Bone marrow aspiration. J Clin Pathol., 2001; 54(9): 657-63.
5. Nanda A, Basu S, Marwaha N. BMB as an adjunct to BMA. J Assoc Physicians India, 2002; 50: 893-5.
6. Amrish Pandya, Tejas Patel, Neelam Shah. Comparative utility of bone marrow aspiration and bone Marrow biopsy. Journal of Evolution of Medical and Dental Sciences, 2012; 1(6): 987-93.
7. Smita Chandra, Harish Chandra. Comparison of bone marrow BMA cytology, BMI cytology and BMB for bone marrow evaluation. Hematology Reports, 2011; 3: e22.

Table - 6: Evaluation of BMA, BMB and BMI based on different parameters.

	BMA	**BMB**	**BMI**
Procedure	Easy and smooth procedure. Very little discomfort to the patient. Dilution of the specimen by blood is common problem with BMA.	Relatively painful procedure. Chances of procedure failure are high and distortion of area in which BMA is done.	If proper cautions are not taken while preparing imprints, chances of breaking down of BMB specimen are there.
Processing and staining	BMAs are cheaper to prepare, rapidly stained with widely available routine stains. Ready for examination within few minutes. Cost effective.	Sections are expensive, preparation & processing requires at least 6-24hrs. Techniques to cut sections with minimal distortion at 4 to 5 µm require experienced and trained person.	Same as BMA.
Cellularity	Approximation of cellularity can be done from direct smear but dilution by peripheral blood makes all types of smears and volumetric data worthless in accurate estimation of cellularity.	It is reliable mode of accurate determination of cellularity	Imprints give good idea of cellularity. But though they are not spread smears, chances of false positive results regarding cellularity is there. In hypercellular marrow imprints may not have cell drop out.
Megakaryocytes	Megakaryocytic density by BMA smears examination is clearly measured. Morphology is excellent.	The megakaryocytes are accurately assessed.	Imprint smears obviate the difficulty of assessing megakaryocytic density as well as morphology.
Topographical alteration of marrow		It is only seen in BMB. Megakaryocytic heterotopia is well observed in PV.	

Differential count	The smears in which marrow material is evenly spread & undistorted. So, this preparation is convenient for differential count. At least 500 cells should be calculated in different particles, this can be easily done on BMA.	Not possible.	The cytomorphology is not so appreciated, so as to do differential count in at least 500 cells.
Morphology	BMA smears are found to be ‘’the best’’ for examination of cytological details since BMA has not been anticoagulated or stored before making smears. By this almost all diagnosis can be made except for IMF, AA and LPD.	Cells get shrunken in this preparation, so cellular details are not satisfactory.	The morphology is relatively less appreciated as the imprints are not spread smears. So over cellular imprints and overlapping of cells may obscure exact cytomorphology.
	Cytomorphology characterization of immature cells (blasts) is better on BMA. So FAB classification of acute leukemia is applied on smear but not on sections.		Immature cells tend to be hidden in overlapped areas of smear, so the ratio of immature to mature cells is artificially depressed in smears
	In smears evaluation of intracellular inclusions, maturation of erythroid precursors are more satisfactory .	Morphological identification of individual cell is more difficult than smear	
	Basophilic granulocytes & precursors are recognizable in Romanowsky stained smears but not in section.		
	Morphology of mitotic figure is better. Because dividing cells are concentrated in particles.		

Iron content	BMA is superior, in estimation of iron stores. Identification of iron store is done only on BMAs.		The particles are too less in imprint material so there are high chances of false negative results.
Pattern of involvement	Easily missed or not apparent.	Only trephine section identifies pattern of involvement which is important for diagnosis and staging of lymphoma, hairy cell leukemia, multiple myeloma and metastatic tumors.	Not apparent
Metastatic tumors	Often tumor cells are irregularly distributed individually/ in groups so difficult to classify from other marrow cells. Metastasis is under diagnosed or misdiagnosed.	Metastatic tumors are more easily found; better classified and even origin of primary can be judged because of architectural and histological patterns displayed in sections especially when BMA found to be normal.	Same as BMA