DOI: 10.7860/JCDR/2014/10015.5278

Original Article

Pathology Section

Floatation of Decalcified Bone Marrow Core Biopsy – A Clue to Marrow Hypocellularity

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ABSTRACT

Background : Examination of bone marrow plays a pivotal role in the practice of haematology. It can be evaluated by three ways – bone marrow aspiration smears (BMA), bone marrow touch imprints (BMI) and bone marrow biopsy (BMB). BMB sections are considered to be the gold standard for assessing overall marrow cellularity.

Aim and Objective: To evaluate the correlation, if any, between bone marrow cellularity and floatation pattern of the core biopsy specimen, after proper decalcification.

Setting and Design: This study was carried out in the Department of Pathology, Institute of Medical Sciences, Varanasi over a period of 26 months.

Materials and Methods: Specimens of BMA, BMI and BMB were collected from 182 cases. The core biopsy specimens were fixed in 10% buffered formalin for 24 hours, and were decalcified in 5% formic acid for 12 hours. The properly decalcified core biopsy samples were then put into adequate-sized container filled with 10% buffered formalin, and floatation pattern was documented.

Statistical Analysis: All the observations were evaluated using simple and basic statistical tools, i.e. sensitivity, specificity, positive predictive value. Chi square test was applied for obtaining statistical correlation i.e. p-value.

Results: Out of 182 core biopsy specimens, 32.4% (n=59) floated, while rests sank. Out of the 59 floating core biopsies, 57 were hypocellular. Seven core biopsies, among 123 specimens that sank, were hypocellular. The sensitivity and specificity of floatation pattern for hypocellular marrow were 89.2% and 99.1%, respectively. A strong correlation (p-value <0.001) between the floatation pattern and bone marrow cellularity was obtained.

Conclusion: Assessment of floatation pattern of properly decalcified marrow core specimen is reliable for assessing marrow hypocellularity.

Keywords: Bone marrow aspiration (BMA), Bone marrow biopsy (BMB), Bone marrow imprint (BMI), Floatation pattern

INTRODUCTION

The role of bone marrow core biopsy (BMB) cannot be underestimated in practice of modern medicine. It is a very cheap, easy and useful diagnostic tool in many haematological disorders involving or infiltrating the bone marrow, as well as many non-haematological disorders known to have bone marrow involvement. The cytomorphological details of individual cellular components are, however, better appreciated in bone marrow aspirate (BMA) and bone marrow touch imprint (BMI) smears, whereas, bone marrow biopsy provides information regarding overall marrow cellularity, the topographical distribution of normal and pathological cellular elements, and degree of fibrosis. Bone marrow biopsy is therefore complementary to bone marrow aspiration smears and marrow imprints in evaluating bone marrow pathology. However, assessment of bone marrow biopsy is a time-consuming procedure, as it has to undergo through fixation, decalcification, dehydration, block preparation, section cutting, slide preparation and staining before being reported.

Assessment of marrow cellularity, many a times, becomes very important clue to diagnosis, especially in cases of aplastic anemia, myelofibrosis and hypocellular myelodysplastic syndromes. All these conditions may lead to dry tap or blood tap in bone marrow aspiration, and poorly cellular bone marrow imprint smears. Evaluation of marrow cellularity, cellular topography and fibrosis then becomes the differentiating features.

The objective of our study was to observe the floatation pattern of core biopsy specimens after fixation and decalcification, and to correlate this finding with marrow cellularity in different bone marrow pathologies.

MATERIALS AND METHODS

Samples of bone marrow aspiration, imprints and biopsy were collected from medical department and paediatric department of Sir Sunderlal Hospital, Banaras Hindu University, Varanasi, India. The period of sampling was from August 2011 to October 2013.

The standard technique [1] was employed for obtaining the aspirate samples using Salah's needle from posterior superior iliac spine. The trephine biopsy [2] was performed using the Jamshidi's needle with the length of the biopsy core between 2.5-3.5 cm. Touch imprint smears were prepared by using the procedure of gentle touch and rolling of biopsy core on the slide. The biopsy core was then fixed, decalcified and finally 2-3 thin sections were made.

All the patients were checked for having any major coagulation disorder before undergoing any procedure.

The slides of BMA and BMI smears were stained with Leishman and Wright-Giemsa methods, while the BMB slides were stained by hematoxylin-eosin and reticulin methods.

Fixation of core biopsy specimen

After collection, each core biopsy specimen was kept in a properly-labelled clean container filled with 10% natural buffered formalin at pH 7.6 for 24 hours for fixation [3].

Decalcification of core biopsy

After proper fixation, each core biopsy specimen was transferred into a container filled with 5% formic acid for decalcification [3], and kept for 12 hours [4].

Observation of floatation pattern

After proper decalcification, each core biopsy specimen was kept into an adequately-sized container filled with 10% buffered formalin for overnight, and the floatation pattern was documented.

Diagnosis	Number of Cases	Floatation pattern	
		Floating	Sinking
Aplastic anaemia	57	57	0
Nutritional deficiency anaemia	36	0	36
Acute leukaemia	34	0	34
Chronic myeloid leukaemia	15	0	15
Multiple myeloma	12	0	12
Myelodysplastic syndromes	10	1	9
Myelofibrosis	7	0	7
Lymphoma	4	0	4
Tuberculosis	3	1	2
Metastatic adenocarcinoma	2	0	2
Polycythemiavera – spent phase	1	0	1
Chronic lymphocytic leukaemia	1	0	1
Total	182	59	123

[Table/Fig-1]: Floatation pattern of core biopsy specimen in different diagnoses.

		Bone Marrow Biopsy Cellularity		TOTAL
		Hypocellular	Normocellular / Hypercellular	
Floatation pattern	Floating	58	1	59
	Sinking	7	116	123
TOTAL		65	117	182

[Table/Fig-2]: Floatation pattern of core biopsy in relation to bone marrow cellularity

If the core biopsy was more or less floating horizontally on the surface of formalin, it was documented to be floating.

If the core biopsy was not lying horizontally, but was lying vertically or obliquely with one end of the core touching the surface of formalin solution, it was again reported to be floating.

If the core biopsy specimen was either lying at the bottom or anywhere in the depth of the fluid column without touching the surface, it was reported to be sinking.

RESULTS

A total number of 182 cases were studied and their BMA, BMI and findings were documented. These findings were compared to the floatation pattern of core biopsy specimen.

In all the 57 cases of aplastic anaemia, the bone marrow biopsies were hypocellular and all the core biopsies floated after decalcification. All the biopsy specimens from cases of acute leukaemia, multiple myeloma, chronic myeloid leukaemia (CML), chronic lymphocytic leukaemia (CLL), lymphoma, nutritional deficiency anaemia, myelofibrosis, polycythemiavera (PCV) – spent phase and metastatic adenocarcinoma were either normcocellular or hypercellular, and sank after decalcification. One case of MDS showed floatation of core biopsy, and finally it was reported as hypocellular MDS. Rest 9 biopsies from MDS cases, which were hypercellular, sank after decalcification. Out of three cases of Tuberculosis, only one case floated, and on histopathology section it displayed extensive caseation necrosis. The remaining two core biopsies from Tuberculosis sank.

In total, out of 182 core biopsy specimens from different haematological and non-haematological disorders, 59 specimens (32.41%) floated after decalcification, and 57 out of the floating biopsies were diagnosed as aplastic anaemia [Table/Fig-1].

[Table/Fig-2] shows 89.2% sensitivity and 99.1% specificity of the floatation pattern for hypocellularity, with a predictive value of 98.3%. A strong correlation ('p' value <0.001, Chi square test) between the floatation pattern and bone marrow biopsy cellularity was obtained.

Repeated attempts of BMA were met with dry tap or blood tap in 46 cases (25.27%), among which 7 were from myelofibrosis, one with metastatic adenocarcinoma, 22 with aplastic anaemia, 4 with multiple myeloma, 3 with MDS, 7 with acute leukaemia and two cases (13.33%) with CML.

DISCUSSION

Examination of bone marrow plays a pivotal role in diagnosing haematological and non-hematological disorders involving the bone marrow. There are three ways of examining the bone marrow - aspiration smears (BMA), core biopsy touch imprint smears (BMI), and trephine core biopsy sections (BMB). BMA and BMI smears are invaluable for assessing cytomorphological details of marrow cellular elements. This is particularly useful for making diagnoses in conditions like leukaemias, lymphomas, nutritional deficiency anaemias, myelodysplasia, plasma cell dyscrasias, metastatic malignancies and bone marrow infections (e.g. tuberculosis, histoplasmosis). BMBx examination, on the other hand, is essential for assessment of bone marrow cellularity, topographical distribution of individual cellular elements within the marrow spaces and degree of marrow fibrosis, which provide important clues to the diagnosis of aplastic anaemia, myelofibrosis, abnormal localization of immature myeloid precursors (ALIP) etc. BMA, BMI and BMBx are, therefore, complementary to each other [5].

Although assessment of cellularity on BMA particle smears is a common practice, BMB section remains the gold standard [2] for the same. But, examination of BMB is time consuming due to its long processing time.

There are disorders like aplastic anaemia, myelofibrosis and hypocellular MDS, which yield only blood (blood tap) or no haematopoietic cellular element (dry tap), even on repeated attempts of BMA, due to poor cellularity of bone marrow. The same occurs with diseases like leukaemias, lymphomas and metastatic malignancies, due to inability to aspirate from tightly packed marrow. The assessment of cellularity, on BMA smears, becomes very difficult in these previously mentioned circumstances. Moreover, the cytological picture of aplastic anaemia and fibrotic phase of myelofibrosis are similar, possessing difficulties in making a diagnosis based upon cellularity in bone marrow aspirate particles.

In our study, we evaluated 182 cases, both with haematological and non-haematological disorders, with their BMA, BMI and BMB. We observed that after proper decalcification, some core biopsy specimens floated, while others sank. This finding was compared with the cellularity of BMB sections to attain a correlation, if any.

Among the 182 cases, in our study, 59 biopsies floated after decalcification and the rest 123 sank. Out of 59 core biopsies which floated, 57 were from aplastic anaemias, one from hypocellular myelodysplastic syndrome (MDS), and one with tuberculosis. In aplastic anaemia, the haematopoietic cellular elements were replaced by adipocytes, resulting in reduced cellular density. The single case of MDS that floated was again hypocellular. The biopsy section from the case with tuberculosis displayed extensive caseation necrosis of the marrow cellular elements. An overall reduced weight: volume ratio of the core biopsy specimens has been assumed as the cause of floatation in all the above mentioned cases.

Among the 123 core biopsies which sank after decalcification, 39 were normocellular, 77 were hypercellular, and only 7 were hypocellular. The biopsies with normocellularity and hypercellularity were from cases like leukaemias, lymphomas, nutritional deficiency anaemias, myelomas etc, and sank due to normal or increased weight: volume ratio of the core biopsy. The 7 cases, which sank, were hypocellular and finally were diagnosed as myelofibrosis. Increased weight: volume ratio of the core biopsy owing to extensive fibrosis of the marrow spaces has been postulated to be the cause.

Considering cellularity of bone marrow biopsy sections as gold standard, we observed 89.2% sensitivity and 99.1% specificity of the floatation pattern for hypocellularity, while the positive predictive value was 98.3%. A strong agreement (p-value <0.001) is observed

between the floatation pattern and BMB cellularity. This novel finding is not documented by any other previous study. Floatation pattern, however, is unable to differentiate between normocellular and hypercellular marrows.

The strong correlation between the floatation pattern of core biopsy and its corresponding cellularity can, therefore, be utilized for early assessment of marrow hypocellularity depending upon floatation pattern, much before preparation of BMB sections. This will be of particular help where cellularity assessment on BMA particles is not possible owing to aparticulate nature of the smears or bone marrow particles exhibiting variable cellularity.

Combining the clinical pictures, peripheral blood values, bone marrow aspirate findings and floatation pattern of the core biopsies, a presumptive diagnosis can be offered in cases like aplastic anaemias, MDS and myelofibrosis, especially when prompt treatment is warranted in critically ill patients.

CONCLUSION

Although bone marrow aspirate particles are widely used for assessing marrow cellularity, bone marrow biopsy sections are considered to be the gold standard for the same, however, processing of biopsy sections are time-consuming, many a times delaying the diagnosis. A strong statistical correlation between the properly-decalcified floatating marrow core biopsy specimens and bone marrow hypocellularity, obtained in our study, recommends the assessment of floatation pattern of core biopsy specimens, after fixation and proper decalcification, in all cases where trephine core biopsy has been performed as a valuable and rapid aid in evaluation of bone marrow hypocellularity. This will be of particular help when bone marrow aspirate smears are unable to provide information about marrow cellularity.

REFERRENCES

[1] Bain BJ. Bone marrow aspiration. *J ClinPathol*. 2001;54:657-63.
[2] Bain BJ. Bone marrow trephine biopsy. *J Clin Pathol*. 2001;54:737-42.
[3] Bain BJ. Bone marrow pathology. 4th edition. *Wiley Blackwell* 2010, UK.
[4] Barbara BJ. Method of fixation, decalcification & paraffin –embedding. In:Bone Marrow Pathology. Barbara J.B, David MC, Irvin AL, Bridget SW. Third Edition,2001.*Blackwell Publication:*475 & 476.
[5] Chandra S, Chandra H. Comparison of bone marrow aspirate cytology, touch imprint cytology and trephine biopsy for bone marrow evaluation. *Hematology Reports*. 2011;3:22.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **May 17, 2014**
Date of Peer Review: **Jul 29, 2014**
Date of Acceptance: **Sep 11, 2014**
Date of Publishing: **Dec 05, 2014**