Prediction of Bone Marrow Cellularity from Aspiration as Compared to Trephine Biopsy

Saran Abdulkader Noralden[a*], Professor Anwar Sheikha[b], Nawsherwan Sadiq Mohammad[c]

[a]M.B.Ch.B. MSc Hematopathology. Ministry of Health, KRG.Iraq
[b]MD, FRCP, FRCPath., FACP University of Mississippi Medical Centre, Jackson, Mississippi, USA
[c]M.B.Ch.B Hawler Medical University, College of Medicine. Nanakaly Teaching Hospital for Blood Diseases. Erbil- Iraq
[a]Email: saranabdulkader@yahoo.com

Abstract

Background: Bone marrow cellularity is an essential and integral part of the bone marrow examination report. Cellularity could be obtained from both bone marrow aspirations and biopsies. Predicting marrow cellularity from aspiration as opposed to biopsy would give the clinician the convenience of an early diagnosis and timely management. In this study, we aimed at knowing the degree of correlation between the bone marrow aspiration cellularity that could be ready within a short period of time to that of bone marrow biopsy cellularity that could take days to have a positive impact on the management, especially for acute blood disorders. Materials and Methods: We collected 200 consecutive bone marrow aspirations from the Nanakaly Teaching Hospital. All the bone marrow biopsy slides belonging to the same group of patients were also collected from the main histology center at Rizgary Teaching Hospital. Five expert hematopathologists were given the chance to report on the cellularity for both the aspirations and the biopsies. The study was performed in sessions, limiting each session to 20 aspirations and 20 biopsies. Cellularity was rated in percentage points of 5 giving the observer the chance to rate the cellularity from 0% to 100%. Results: Microsoft Excel spreadsheet was used to record all the data obtained from the observers. Mean values from all the five observers for each aspiration and biopsy was used for statistical analysis.

----

* Corresponding author.

We found a strong direct positive correlation between the bone marrow biopsy cellularity and bone marrow aspiration cellularity. Conclusion: A simple practical equation could be created to measure bone marrow biopsy cellularity from the usually available aspiration cellularity. Marrow biopsy cellularity was found to be 0.96 of the aspiration cellularity.

Keywords: Bone marrow; Bone marrow examination; Bone marrow aspiration; Bone marrow biopsy; Bone marrow cellularity.

1. Introduction

Specimens of the bone marrow for cytological details and histological examination may be obtained by aspiration of the bone marrow fluid, by core biopsy using a trephine needle or rarely by open biopsy [1]. The two most important techniques, which are complementary, are bone marrow aspiration and trephine bone marrow biopsy [1,2]. Generally speaking, bone marrow aspiration is easy to perform with minimal discomfort to the patient [1]. It is usually performed on an outpatient basis. Patients should be reassured about the safety of the procedure. All what is needed from the patient is a few drops of marrow, spread professionally on few glass slides. In contrast to the bone marrow aspiration, bone marrow trephine biopsy is more painful and more time consuming [1,3]. While it is the norm in the developed world to perform both procedures together, it is unfortunate in our practice to see lack of complementing bone marrow aspiration procedures with biopsy! Different reasons are given to this limitation; including lack of resources like bone marrow biopsy needles; longer time needed for the report to be ready, often when diagnosis has already been made and treatment has been started; and inadequate number of expert pathologists proficient in reporting marrow biopsies. With such an invasive and painful procedure, repetition of the bone marrow is not justified when enough information cannot be obtained from the aspiration alone. The constraint of doing bone marrow aspiration alone, rather than combined aspiration and biopsy, puts the patients in developing world in a difficult situation. To improve on the health system to the extent of imposing a bone marrow biopsy examination on every patient subjected to bone marrow examination cannot be overemphasized. Troubled and held back by this lack of investigational tool, are we justified to predict the biopsy results from the aspiration alone? We tried to tackle this problem through one of the most important parameters in bone marrow examination, that is the marrow cellularity [2,3,4]. Bone marrow cellularity is a vital component of both aspiration and biopsy, with biopsy being the gold standard. It is our aim to find out if bone marrow aspiration cellularity could be depended upon to predict bone marrow cellularity. A strongly positive correlation would justify starting management based on aspiration alone well ahead of the time biopsy results will be available [1,4,5,6].In this study, we evaluate the bone marrow aspiration cellularity in patients with different hematological diseases. Five independent experienced hematopathologists appraised the aspiration cellularity according to international standards. This was then compared with the more objective trephine bone marrow biopsy cellularity, appraised again independently by the same hematopathologists.

1.1. Materials and Methods

This study was performed on patients admitted and managed in Nanakaly Hospital for Blood Diseases. All the

bone marrow aspirations performed over a two year period (June 2016 to June 2018) were subjected to this investigation. As bone marrow biopsy examinations are sent and reported in Rizgary Teaching Hospital, every bone marrow trephine biopsy belonging to the group of patients were traced, collected and subjected to parallel analysis. In total, 200 consecutive bone marrow aspirations and 200 bone marrow biopsies were analyzed for cellularity estimation. A table was prepared with the numbers of the bone marrows, from 1 to 200, on the vertical axis and cellularity on the horizontal axis. Each bone marrow could be rated on 5% increments from 0% to 100%. Five expert pathologists were chosen to rate the cellularity on each aspiration. Each observer was given the chance to report the cellularity after the leading microscopist was showing and navigating the slide for a minute, controlled through a stop watch. The examiners used to rotate between themselves as the leading microscopist. After completion of the bone marrow cellularity, the same procedure was repeated for the corresponding bone marrow biopsies. The whole exercise took 20 days to complete. To avoid the fatigue factor, each day no more than one session of two hours was allowed. It was a labor intensive commitment, especially taking into consideration resorting to expert pathologists from many different hospitals. Each examiner provided 400 results, 200 for marrow aspirations and 200 for bone marrow biopsies. The slides were first examined briefly by naked eyes to asses its thickness and quality. The bone marrow aspiration cellularity was reported upon based on the overall cellularity that included both the fragments "spicules" and the trails. On few occasions, the fragment cellularity did not match with the trail cellularity. The priority was given to the fragment cellularity in solidly cellular fragments with hypocellular trials. When fragments were of normal or lower cellularity but the trails were cellular, the trail cellularity was taken into consideration as well [7].   As for the detailed estimation of bone marrow aspiration cellularity, the evaluation was very subjective as no single rule could be applied. An overall impression was made based on the crowdness of the fields, fat cell content of the fragments and trail overall cellularity. Bone marrow biopsy cellularity, a gold standard for true marrow cellularity, was far easier based on proportion of fat to hematopoietic cells [7,8]. To avoid irregularities in regard to the timing of each slide inspection, it was agreed to time the event for each slide to one minute. Examiners were only allowed extra time for complicated cases and based on group agreement. Slides were first scanned using the 4X scanning objective lens with the 10X eye-piece lens. Marrow biopsies were then screened with the 10X and 20X objectives. As for the aspiration cellularity estimation, the same rules applied but on occasions the 100X oil immersion lens was supplemented the cellularity assessment [8]. The observers were completely blind as to which aspiration belongs to which biopsy. This was aided by the fact that all the aspirations were evaluated first and then the biopsies. Data obtained were subjected to statistical analysis with emphasis on correlation between each bone marrow aspiration cellularity and its matching biopsy cellularity. Each observer and inter-observer values were critically analyzed. Results from statistical analysis, using Statistical Package for Social Sciences (SPSS Version 23.0), was used to obtain regression between cellularity for each individual bone marrow aspiration and its biopsy cellularity. Results obtained through t test was used to compare between bone marrow aspiration and biopsy cellularity readings. Graphs and Regression equations were obtained and calculated using SPSS.

1.2. Results

Graphic representation of the results provided by each observer and the means of all the observers are seen in Figures 1 to 6. It was noted that there was an excellent degree of correlation between the five observers for each

individual aspiration and each individual biopsy evaluation. Analyzing the graphs for each observer and the means of all the samples comparing the bone marrow aspiration cellularity with its *associated* bone marrow trephine biopsy cellularity, significant correlation was observed. Few readings, about four to be exact, however, showed a big drift between the two cellularities. Those drifts were mostly reproducible between the observers, indicating genuine variability in the bone marrow cellularity, especially in the aspirate, since biopsy cellularity is the gold standard for cellularity estimation. This occasional drift is understandable when it is translated to clinical terms like in patients with patchy marrow cellularity when the needle hits a hypocellular area, whereas the biopsy gives a true representation for the overall cellularity. Other possible explanations could be in cases of acute myelofibrosis, chronic myelofibrosis or hairy cell leukemia or even some acute leukemias when the blast cells are very sticky and defy release and proper representation in the bone marrow aspirations. Out of 200 marrow samples, such phenomena should be expected.

1.3. Statistical Analysis

We subjected our data to vigorous statistical analysis utilizing SPSS version 16.0, also it was used for plotting the graphs and calculating the regression equation. t test was used to compare between aspiration and biopsy readings. The aim was to find out if aspiration result could replace biopsy. The p-value obtained was 0.199, indicating a non-significant difference between the two readings, i.e. both are so close that no difference could be detected. We applied Correlation and Linear regression model to the "means" of 200 bone marrow aspiration cellularities and their 200 associated bone marrow biopsy cellularities. The "means" of the five observers were used for each specimen. The aim was to investigate the linear association between the two continuous variables, the bone marrow aspiration and their biopsy cellularity. Correlation is supposed to measure the closeness of the association. Linear regression is supposed to give the equation of the straight line that best describes this closeness of association and enable the prediction of the bone marrow biopsy from the bone marrow aspiration cellularity. For the calculation of the correlation, the bone marrow biopsy cellularity was given the notation of (X) and the bone marrow aspiration cellularity was given the notation of (Y). As seen in table 1, the parameters needed for the statistical analysis were:

Table 1: Parameters of statistical analysis

1	Bone Marrow Biopsy Cellularity	X
2	Bone Marrow Aspiration Cellularity	Y
3	Square of each Biopsy "mean"	X^2
4	Square of each Aspiration "mean"	Y^2
5	Sum of all the Biopsy "means"	ΣX
6	Sum of all the Aspiration "means"	ΣY
7	Sum of all the X^2	ΣX^2
8	Sum of all the Y^2	ΣY^2
9	Sum of all the "XY"s	ΣXY
10	Square of ΣX	$(\Sigma X)^2$
11	Square of ΣY	$(\Sigma Y)^2$

Table 2 shows statistical tabulation of data for final evaluation of correlation.

Table 2: Statistical tabulation of data for final evaluation of correlation

Number	Biopsy Cellularity X	X^2	Aspiration Cellularity Y	Y^2	XY
1					
2					
3					
4					
..					
…					
….					
˜200					
	ΣX	ΣX^2	ΣY	ΣY^2	ΣXY

In general bone marrow aspiration cellularity tends to be associated with bone marrow biopsy cellularity. This association could be measured by Pearson correlation coefficient (r):

$$r = \frac{\sum (X - \bar{X})(Y - \bar{Y})}{\sqrt{[\sum (X - \bar{X})^2 \sum (Y - \bar{Y})^2]}}$$

r= 0.720

Figure 1 shows the regression line obtained for the bone marrow biopsy cellularity against the bone marrow aspiration cellularity. From the graph, it is evident that there is a high degree of correlation between the two cellularities.

Results obtained are as follows:

N = 200

ΣX = 13200   Mean X = 66

ΣX^2 = 949912

ΣY = 13689   Mean Y = 68.445

ΣY^2 = 1002273

To validate the significance of the correlation, a t test was used to test whether (r) is significantly differing from

zero i.e. whether the observed correlation could simply be due to chance. The p-value obtained was 2.943 E-33, indicating a highly significant relation, i.e. concordance, of both the aspiration and biopsy cellularity readings. Note: A significant level is a function of both the size of the correlation coefficient and number of observations. A weak correlation may therefore be statistically significant if based on a large number of observations, while a strong correlation may fail to achieve significance if there are only a few observations. Statistically, we needed 27 aspirations and biopsies to reach a conclusion; we chose 200 samples to have an infallible proof for correlation or lack of it.

Interpretation of r:

r is always a number between minus 1 and plus 1.

r is positive if X and Y tend to be associated with each other, and the larger its value, the closer is the association. Table 3 shows the detail.

Table 3: Interpretation of r

r value	Interpretation
Zero	No correlation
0 to 1	Direct positive correlation
1	Perfect direct positive correlation

Table 4: Shows degree of association based on value of r**.**

R	Degree of association
+1	Perfect direct association
+0.7 to +1	Strong direct association
+0.4 to +0.7	Moderate direct association
+0.2 to + 0.4	Weak direct association
+0.1 to +0.2	Negative direct association
0.0	No association

Using the regression line an equation could be used to estimate bone marrow biopsy cellularity from the bone marrow aspiration cellularity.

Normal P-P Plot of Regression Standardized Residual

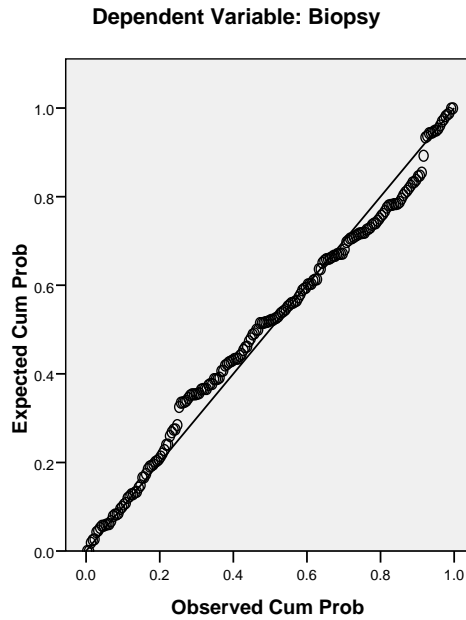**Dependent Variable: Biopsy**

Figure 1: Regression line Fitting the data of the mean with intercept

So we tried to fit the same model but this time without the intercept term in order to increase the R square. The following table shows the estimates of the parameters:

Table 5: Coefficients(a)

Model	B	P-Value
Aspiration	0.956	0.000

a Dependent Variable: Biopsy

the fitted model is as follows:

Biopsy = 0.956 Aspiration

This means that every one unit in Biopsy is equivalent to a 0.956 in Aspiration. It is noted that the model selection in this manner is the best since the p-value concludes that it is highly significant.

Normal P-P Plot of Regression Standardized Residual

Dependent Variable: Biopsy
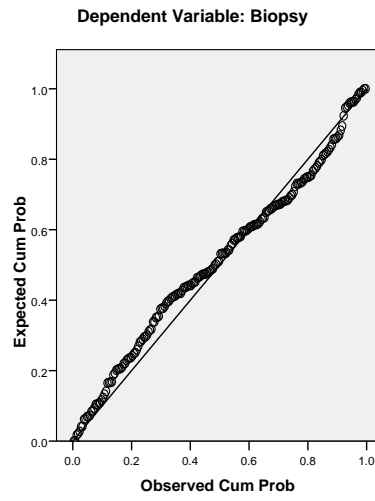
Figure 2: Regression line fitting the data of the mean without intercept

The regression line fitting the five observers individually are shown in figures 3-7.

Normal P-P Plot of Regression Standardized Residual
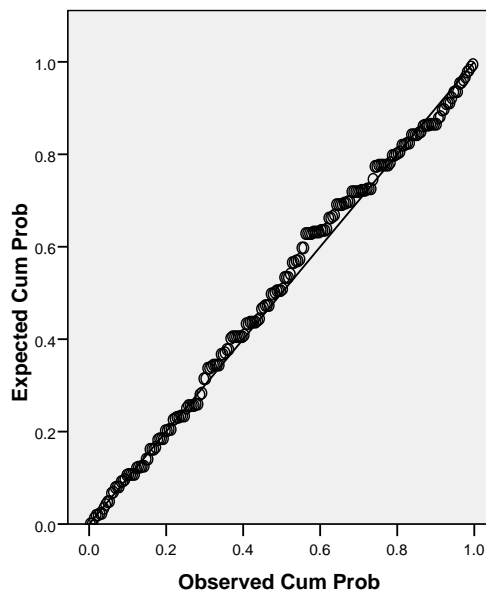
Dependent Variable: Dr. First Observer Biopsy

Figure 3: Regression line Fitting the data of observer one without intercept

Normal P-P Plot of Regression Standardized Residual
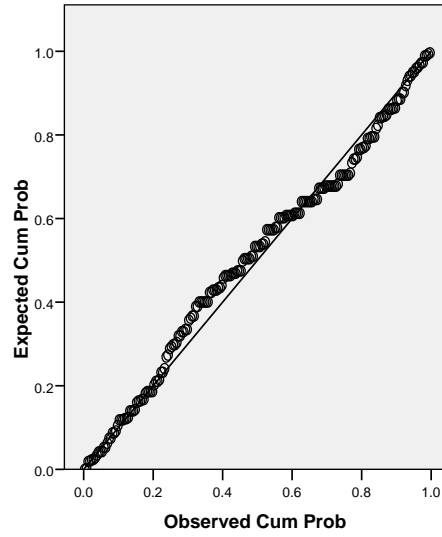
Dependent Variable: Second Observer Biopsy

Figure 4: Regression line Fitting the data of observer two without intercept

Normal P-P Plot of Regression Standardized Residual
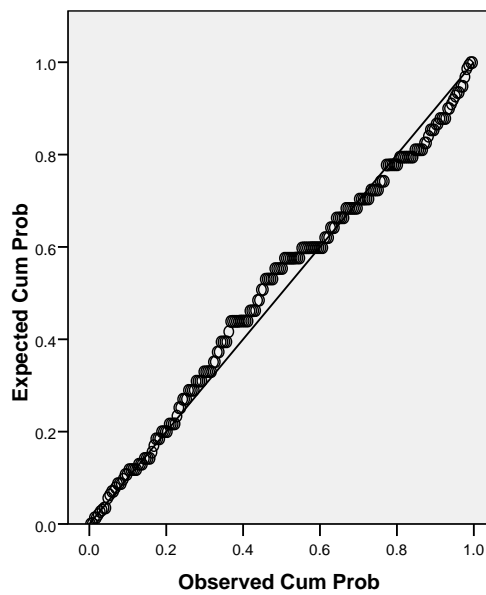
Dependent Variable:Third ObserverBiopsy

Figure 5: Regression line Fitting the data of observer three without intercept

Normal P-P Plot of Regression Standardized Residual

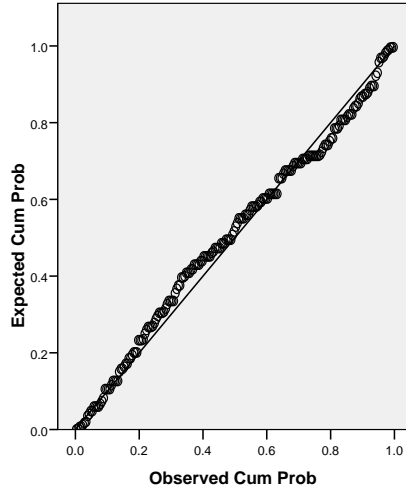**Dependent Variable: Fourth Observer Biopsy**

Figure 6: Regression line fitting the data of observer four without

Normal P-P Plot of Regression Standardized Residual
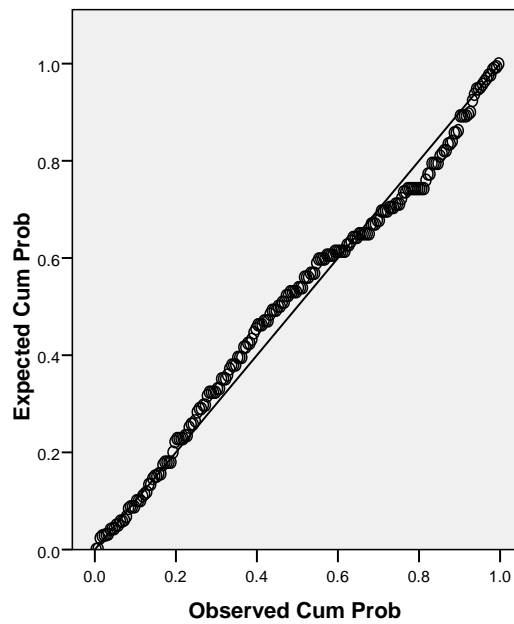
Dependent Variable: Fifth Observer Biopsy

Figure 7: Regression line Fitting the data of observer Five without intercept

2. Discussion

Performance of bone marrow biopsy in an overweight patient could be extremely difficult; extrapolation of the cellularity from the aspiration alone could be detrimental in making the proper diagnosis [9,10]. In the developing and underdeveloped world access to bone marrow biopsy is even more limited because of lack of pathologists, need for a proper histopathology departments and conflict of interest between hematopathologists and histopathologists. While aspiration could be done and processed by the same team, histology is usually part of a different department with bone marrow biopsy constituting only a fraction of their daily routine. Biopsy needs decalcification, embedding in a block, microtome cutting and specialized staining, all tedious time-consuming processes. Aspiration is a straightforward handy procedure that needs marrow aspiration and immediate Romanowsky staining [7,11]. Cell morphology is far better delineated on aspiration than biopsy and most blood diseases could confidently be diagnosed on aspiration alone. Extra cost and effort associated with performing bone marrow biopsy are few more limitation factors for not doing biopsy with every aspiration. Most private laboratories and peripheral hospitals can do and stain bone marrow aspirations but have no access to in-situ proper surgical pathology laboratories [12]. Most of the time the clinicians need urgent results that jeopardizes depending on the biopsy back-up results and that entails releasing the result of bone marrow aspiration far before the results of biopsy is available. Last, but not the least, there is a definite split of interest throughout most of the world regarding the allocation of processing and reporting of the bone marrow aspiration and biopsy. Although in North America, they could both be within the domain of the hematopathology, in Europe and most of the world they are processed in completely two different departments, hematology and histopathology. That could well be translated to a delay in the availability of the bone marrow biopsy result [8]. From the above accounts, one can easily say that by the time the results of bone marrow trephine biopsy is available, the diagnosis has already been made and management has already begun. In many medical specialties, confusion on the part of the clinicians as to the true meaning of aspiration and biopsy adds yet another dilemma into the exact need for biopsy as part of the bone marrow procedure. It is not unusual for the clinician to ask for marrow examination without having comprehension regarding the fact that there are two items in bone marrow examination, aspiration and biopsy [13]. In this study we came to the firm belief that our shortcoming of not having easy access to bone marrow biopsy cellularity is not a detrimental factor in the management of our patients. With such a high concordance rate between the observers, bone marrow biopsy cellularity, the gold standard for true bone marrow cellularity, can be comfortably predicted from bone marrow aspiration cellularity. Expressing such a reassuring opinion, however, should be cautious, since there are odd situations when the marrow aspiration is not quite representative. Four of our marrow aspirations showed a big drift between the two cellularities! Since it was reproducible between all the observers, we reached to the conclusion that the bone marrow aspirates were not representative for the biopsies. That means that the above significant association between the bone marrow aspiration cellularity and biopsy cellularity is only valid when both the aspiration and biopsy are performed and stained properly and professionally. Dilute, dry and difficultly-aspirated or clotted marrow aspirations should be complemented with proper biopsy for correct evaluation of cellularity [14]. We are not aware of such a study in literature on such a high number of cases or with similar intentions. Reaching to a statistically-approved and ANOVA-evaluated equation seems to be a historical landmark. To be able to estimate biopsy cellularity through a simple equation based on aspiration cellularity is

beneficial to the patient, cost-effective on health resources and reassuring to the clinician. Now we can confidently apply the equation that biopsy cellularity is equivalent to 0.96 of Aspiration cellularity in percentage points, i.e., a bone marrow aspirate of 60% cellularity is supposed to have a biopsy cellularity of 58%. In conclusions; Bone marrow aspiration cellularity is representative of bone marrow biopsy cellularity when the marrow aspiration is representative of the marrow pathology and the marrow specimen is not clotted, dilute, dry or difficult and Clinicians managing patients with hematological diseases can start managing their patients without undue delay for the biopsy result to be available. Compliance with Ethical Standards Conflict of interest: The authors declare that they have no conflict of interest. Ethical Approval: Ethical approval has been done by the ethical committee of medical college Hawler Medical University

References

[1]. Babyn PS, Ransom M, McCarville ME et al. Normal bone marrow: signal characteristics and fatty conversion. Magnetic resonance imaging clinics of North America 1998 6(3): 397-405.

[2]. Bain B, Clark DC, Lampert IO, Wilkins BS. Bone marrow pathology, 3rd ed., 2000 Blackwell Sciences, Oxford.

[3]. Bain BJ. Bone marrow aspiration. Journal of Clinical Pathology (2001a), 54: 657-663.

[4]. Bain BJ. Bone marrow trephine biopsy. Journal of Clinical Pathology (2001b), 54: 737-742.

[5]. Bain BJ. Bone marrow biopsy morbidity and mortality. Br J Haematol. 2003 Jun;121(6):949-51.

[6]. Emani M, Malhotra S, Rana F. Bone marrow aspirate showing Histoplasma capsulatum. Blood. 2014 Feb 13;123(7):957.

[7]. Lewis S, Bain BJ, Bates I. Dacie and Lewis Practical Haematology. 11th ed. UK. Churchill Livingston 2011. PP 115-127.

[8]. Lee SH, Erber WN, Porwit A, Tomonaga M, Peterson LC; International Council for Standardization In Hematology. ICSH guidelines for the standardization of bone marrow specimens and reports. Int J Lab Hematol. 2008 Oct;30(5):349-64.

[9]. Devaliaf V, Tudor G. Bone marrow examination in obese patients. British Journal of Haematology 2004, 125:537-539.

[10]. Konda B, Pathak S, Edwin I, Mishall P, Downie SA, Olson TR, Reed LJ, Friedman EW. Safe and successful bone marrow biopsy: an anatomical and CT-based cadaver study. Am J Hematol. 2014 Oct;89(10):943-6.

[11]. Bain BJ. Morbidity associated with bone marrow aspiration and trephine biopsy - a review of UK data for 2004. Haematologica. 2006 Sep;91(9):1293-4.

[12]. Minnema MC, de Keizer B. False-positive PET scan after bone marrow biopsy. Br J Haematol. 2013 Jun;161(6):753.

[13]. Wickramasinghe S, McCullough J. (2003). Blood and Bone Marrow Pathology. 1st ed. UK. Churchill Livingstone.

[14]. Engeset A, Nesheim A, Sokolowski J. Incidence of dry tap on bone marrow aspirations in lymphomas and carcinomas: diagnostic value of the small material in the needle. Scandinavian Journal of Haematology 1979, 22: 417-422