Original Article

Correlation of Bone Marrow Aspiration, Touch Imprint Findings and Bone Marrow Biopsy Findings in Pancytopenia.

R. Sindhu*, Pramita Sahu, Debi Prasad Mishra, Samir Kumar Behera

Dept. of Pathology, MKCG Medical College, Berhampur, Odisha, India

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ABSTRACT

Background: Pancytopenia is an important hematological entity encountered in our day to day clinical practice.Bone marrow examination is a useful and cost effective diagnostic approach in evaluating these disorders. Although bone marrow aspiration alone is sufficient in diagnosing most of these disorders, the comparative evaluation of bone marrow aspiration (BMA), imprint (BMI) and biopsy (BMB) is needed for more rapid and efficient diagnosis. Many studies have been done in evaluating the importance of each of these procedures in various hematological disorders, with very few studies evaluating the importance of all three procedures in pancytopenia specifically.So, the objective of our study was to compare and correlate all three procedures in cases of pancytopenia.

Methods: All patients who were found to be pancytopenic were evaluated and bone marrow aspiration, imprint and biopsy were done in all the cases simultaneously at the same site using two needle technique.

Result: A total of 148 cases of pancytopenia were studied and most common cause of pancytopenia was hypoplastic/ aplastic anemia (33.8%), followed by nutritional anemia (30.4%). We found a positive correlation of 72.04% in BMA with BMB and 91.20% in BMI with BMB.

Conclusion: Trephine biopsy has greater value in providing information about the architecture, pattern of cellularity, and presence of infiltrates and granulomas. At the same time morphological features of individual cells may be identified by aspiration and by imprint in cases of dry tap. Hence, both BMA and BMB along with imprint cytology are complementary to each other.

***Corresponding author:**
Dr. R. Sindhu, Department of Pathology, M.K.C.G Medical College, Berhampur, Odisha 760004
Phone: +91 - 7854831852
Email: Sindhushankar51@gmail.com

Introduction

Pancytopenia is an important hematological entity encountered in our day to day clinical practice. A spectrum of disorders, primarily or secondarily affecting the bone marrow may manifest with pancytopenia.[1] Evaluation of the bone marrow is an indispensable adjunct to the study of hematopoietic disorders. Marrow can be examined by aspiration, or trephine biopsy and preparing a clot section from aspiration material orpreparing imprint smears from biopsy. Each procedure has its own advantages and disadvantatges. The present study was therefore conducted to compare and correlate the role of bone marrow aspirate cytology, touch imprint cytology and trephine biopsy sections to formulate an effective and rapid method for diagnosing the hematological and non hematological causes of pancytopenia.

Material and Methods

A prospective study was done in our department. All patients who were found to have Hb <10 gm/dl, TLC < 4.00x103 Cells/µl and platelet count <100x103 cells/µl [2] (done through automated five part cell analyzer Sysmex xt-2000i) were considered to be pancytopenic and included in our study. Diagnosed patients who are already under chemotherapy were excluded from the study. After getting a written consent from the patient both bone marrow aspiration and biopsy were done simultaneously (biopsy being done first) using 2 needle technique at the same skin incision site. A trephine length of 1.5 – 2cm was considered to be adequate. At least 4 good smears were prepared from the aspirate material. Imprint smears were also prepared by touch and roll technique in all cases before fixation of biopsy core. Special stains like Perl's stain for iron, reticulin stain, PAS, MPO, and CAE were done wherever required. The bone marrow aspiration and biopsy were reported according to the ISCH Protocol.[3] Both touch imprint and aspiration were correlated with trephine biopsy. The findings were tabulated in MS Excel 2010 and analysed using the statistical software InStat Graph Pad (Trial Version). Some of the distinct cases with special stains are shown in the figure (figure 1 to 6).

Result

A total of 148 cases of pancytopenia of varying ages were included in the study. Most of the cases affected were in the age group of 11-20 years (22.29%). The most common presenting clinical feature was pallor (98%), followed by easy fatiguability, fever, hepatomegaly, splenomegaly, lymphadenopathy, bleeding tendency, petechiae, sternal tenderness.Most of the cases were non-neoplastic (74.3%), neoplastic ones constituted 25.6% of total cases. Most common cause of pancytopenia in our study was hypoplastic/aplastic anemia (33.8%), followed by nutritional anemia (30.4%), acute leukemia (11.4%), MDS (6.08%), metastatic carcinoma (2.7%), MPN (2.7%), malaria (2.7%), multiple myeloma (2.02%). Other rare causes include primary lymphoma of bone marrow, hemophagocytic lymphohistiocytosis, storage disorders, granulomas. In contrast, most common cause of pancytopenia in children <10 years was found to be ALL. We also found 10 cases of dry tap in BMA where BMB and imprint helped in diagnosis. Imprint was inadequate/not suitable in 4 cases and biopsy was inadequate in 10 cases.

The various cause of pancytopenia diagnosed through each procedure is tabulated in detail (TABLE 1). We also

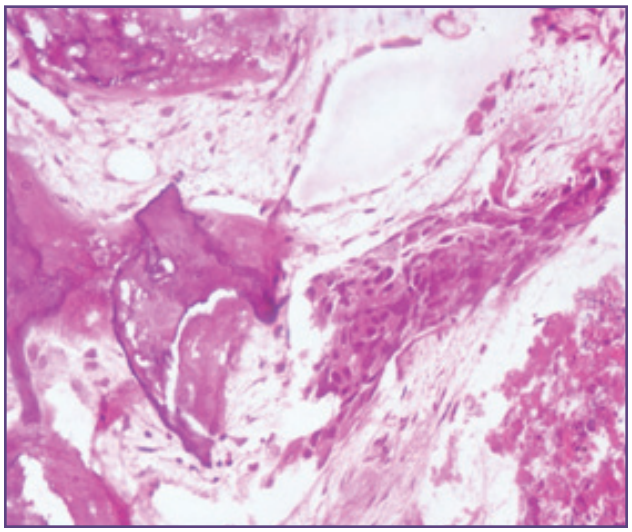

Fig. 1: Microphotograph of BMB in metastatic squamous cell carcinoma from carcinoma cervix.H&E (X400x).

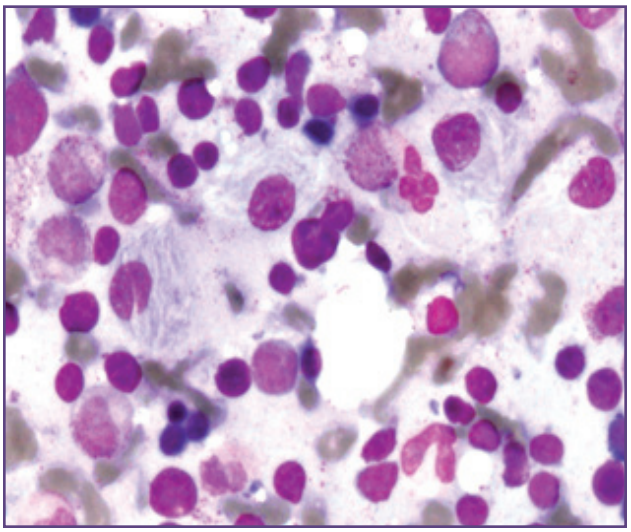

Fig. 2: Microphotograph of BMA in Gaucher's disease. Leishman Stain (X1000x).

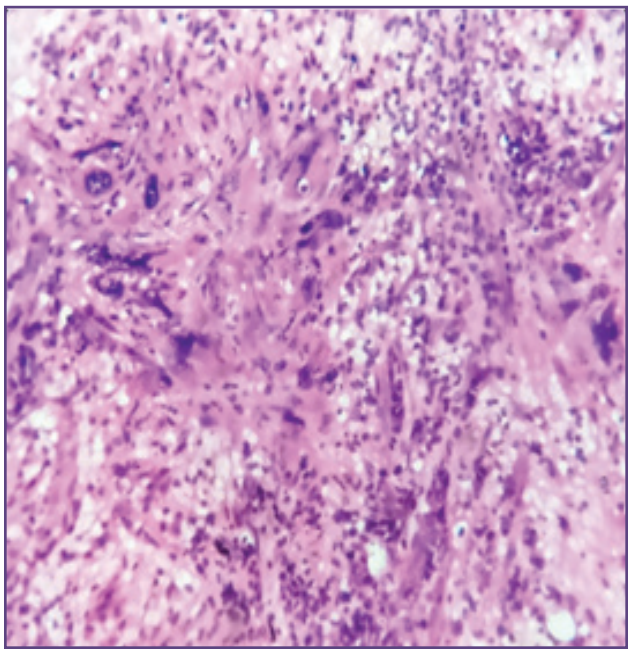

Fig. 3: Microphotograph of BMB and in myelofibrosis. H&E(X400x).

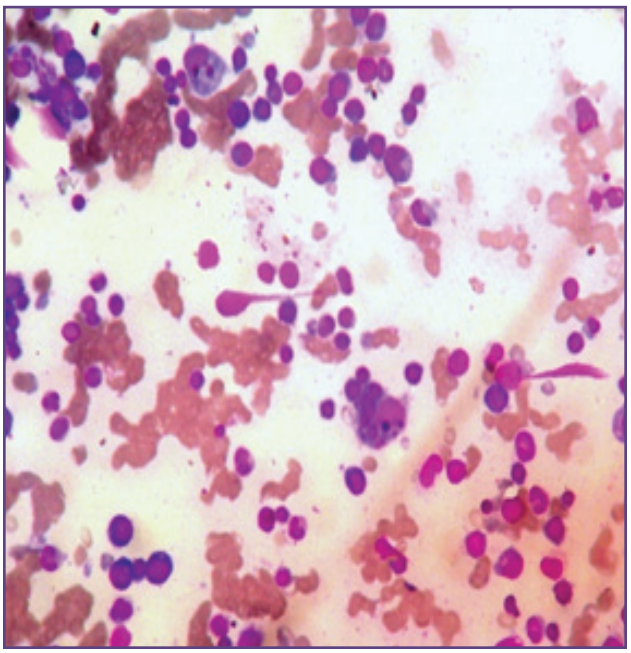

Fig. 4: Microphotograph of BMA in malaria with hemophagocytosis. Leishman Stain (X100x).

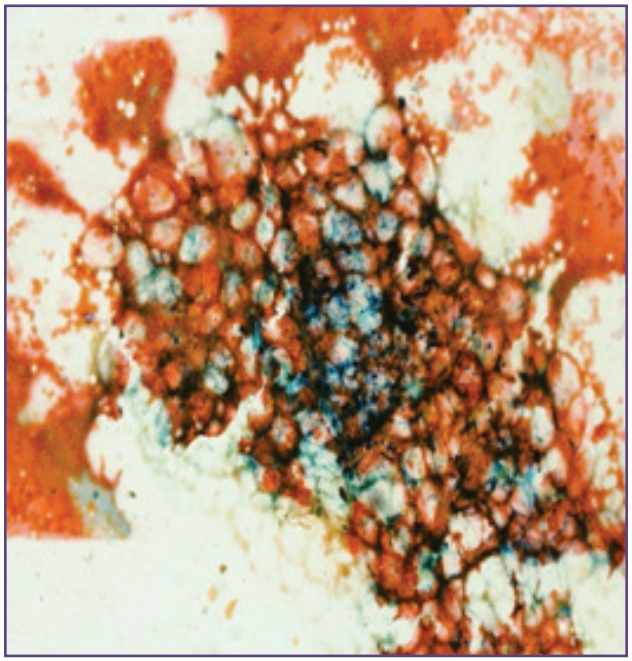

Fig. 5: Microphotograph of BMA showing Grade 4 iron stores in megaloblastic anemia.Perl's stain (X400x).

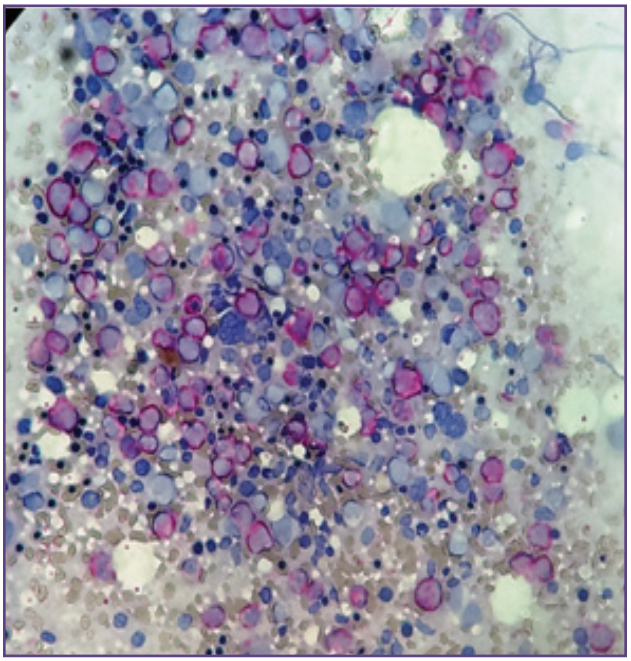

Fig. 6: Microphotograph of BMI in MDS Chloracetate Esterase Stain.(X100x).

correlated both aspiration and imprint with trephine biopsy (TABLE 2). We found a positive correlation of 72.04% in BMA with BMB and 91.20% in BMI with BMB. We were unable to correlate aspiration and imprint with biopsy in certain cases like nutritional anemia, malaria and some miscellaneous cases because biopsy material was inadequate and aspiration and imprint was more helpful in diagnosing those cases.

Discussion

To report a reliable diagnosis, an adequate biopsy material is the most important one.It depends upon various factors

Table 1: CASES DIAGNOSED ON BMA, BMI, BMB

S. NO	CAUSES	TOTAL CASES	BMA DIAGNOSIS	BMI DIAGNOSIS	BMB DIAGNOSIS
1	Nutritional anemia	45 (30.4%)	45	45	43
2	Hypoplastic/aplastic anemia	49 (33.1%)	39	49	49
3	Acute leukemia	17 (11.4%)	13	17	17
4	Myelodysplastic syndrome	09 (6.08%)	05	05	09
5	Multiple myeloma	03 (2.02%)	2	03	03
6	Metastatic carcinoma	04 (2.70%)	02	03	03
7	Myeloproliferative neoplasms	04 (2.70%)	1	01	04
8	Lymphoma	01 (0.6%)	00	00	01
9	Hemophagocytic disorders	02 (1.35%)	02	02	02
10	Storage disorders	01 (0.6%)	01	01	01
11	Granulomatous lesion	01 (0.6%)	00	00	01
12	Malaria	04 (2.70%)	04	04	03
13	Normal bone marrow	02 (1.35%)	12	02	02
14	Dry tap/ scanty aspirate	---	10	---	----
15	Inadequate biopsy/imprint	---	---	10	04
16	Miscellaneous causes	06 (4.05%)	06	06	05
	TOTAL CASES	**148 (100%)**	**148**	**148**	**148**

Table 2: CASES SHOWING POSITIVE CORELATION OF BMA AND BMI WITH BMB

S. NO	CAUSES	TOTAL CASES	Positive correlation of BMA cytology and BMB (% of cases)	Positive correlation of BMI cytology and BMB (% of cases)
1	Nutritional anemia	45	----	----
2	Hypoplastic/ aplastic anemia	49	39 (79.5%)	49 (100%)
3	Acute leukemia	17	13 (76.4%)	17 (100%)
4	Myelodysplastic syndrome	09	5 (56.5%)	5 (56.5%)
5	Miscellaneous causes	06	----	----
6	Multiple myeloma	03	2 (66.66%)	3 (100%)
7	Metastatic carcinoma	04	2 (50%)	3 (75%)
8	Myeloproliferative neoplasms	04	1 (25%)	1 (25%)
9	Lymphoma	01	0	0
10	Hemophagocytic disorders	02	2(100%)	2 (100%)
11	Storage disorders	01	1(100%)	1 (100%)
12	Granulomatous lesion	01	0	0
13	Malaria	04	----	----
14	Normal bone marrow	02	2(100%)	2 (100%)
	TOTAL % OF POSITIVE CORRELATION	**148**	**67 (72.04%)**	**83 (91.20%)**

like the person who does the procedure, the technique used, the site of biopsy etc. We used *Islam's two needle technique*[4] for all our procedures. Both BMA and BMB were done at the same skin incision site with change of the position of the needle after one procedure to get the maximum material as also done by *Pampa ch Toi et al.*[5] Around 97.2% of our biopsy samples were found to be adequate with the criteria for adequacy considered as average length of biopsy core to be 1.5 cm or specimen which shows at least 10 partially preserved intertrabecular areas, likewise *P. T. Dambhareaet al.*[6]who found 96. 55% adequacy.

In the present study, we had 10 cases of (6.75%) dry tap out of which 2 were metastatic carcinomas to bone marrow (1 from cervix, 1 from unknown primary), 1 came out to be primary non Hodgkin lymphoma of BM, 4 cases of acute leukemia presenting as dry tap because of packed marrow and 3 cases of myelofibrosis in late stage (with reticulin stain showing GRADE 3 fibrosis in all the cases). All these cases were diagnosed by BMB. Thus, all the 10 dry tap cases in aspiration were found to be abnormal in biopsy. This was in accordance with the study done by ***Navone R et al.***[7] and***AmrishPandya et al.***[8] who also found 100%

abnormal biopsy in dry tap cases. But study done by ***Engest A et al.***[9]and***Humphries JEet al***.[10] found only 77%, 93% abnormalities in their studies respectively.

In the present study 10 cases of hypoplastic anemia were diagnosed by biopsy which were misdiagnosed as normocellular marrow in BMA. This was in accordance with other studies done by ***P. Ch Toi et al.,***[5]***Vidisha et al.,***[11] ***Patel Set al.,***[12]***Niranjan et al.***[13] who observed that this discordance is because of dry tap in aspiration, or dilution with peripheral blood or aspiration of the hematopoietic foci yielding a normocellular marrow which may be the cause in our study. ***Cetto et al***[14] observed that most of the hypercellular marrows are better identified in BMA as hypercellular but discordance occurs in interpretation of normocellularity and hypocellularity.

Coming to myelodysplastic syndromes, in the present study 4 out of 9 cases were diagnosed by biopsy which was misdiagnosed as megaloblastic anemia in BMA. It is a well-known fact that topography is assessed by biopsy and ALIP which is specific for MDS is assessed only through biopsy which was also observed by ***Gupta N et al***.[15]

Out of 17 cases of acute leukemia 4 cases were diagnosed by BMB. The reason for this may be due to extreme cellularity and compactness of marrow as observed by ***Niranjan et al.***[13] According to ***Bird AR et al.***[16]marked increase in reticulin also leads to dry tap in acute leukemia.

Out of 4 cases of MPN, 3 cases were myelofibrosis which were diagnosed by biopsy and 1 case was mastocytosis diagnosed in BMA and confirmed by BMB. The diagnosis of myelofibrosis needs BMB with reticulin grading in BMB as BMA is dry tap in almost all cases.

A single case of multiple myeloma was diagnosed in BMB which was misdiagnosed as simple reactive plasmacytosis in BMA. The discordance is because identification of sheets of mature and immature plasma cells is more in favor of multiple myeloma than a reactive condition which was observed by ***Sabharwal BD et al***.[17]

A single case of primary Non-Hodgkin lymphoma of bone presenting as pancytopenia was diagnosed through BMB. A study done ***by Gupta N et al.,***[15] ***Menon NC et al.,***[18] observed that bilateral biopsy is required for diagnosis of NHL since unilateral biopsies can miss the diagnosis. But in our study we found only a single case of Non Hodgkin lymphoma which was diagnosed with unilateral biopsy. According to ***Ellman L.***[19] the reason for dry tap in cases of lymphoma are, that nodules and cluster of lymphoma cells can be quite dense and adherent and may be difficult to aspirate. A study done by***SurbhiGhoyalet al***.[20] found that lymphoma diagnosis sensitivity increases with trephine length.

2 out of 4 cases of metastatic carcinoma wasdiagnosed through BMB where BMA was dry tap. This again reiterates the importance of biopsy in cases of dry tap as also observed by other studies.Whereas a single case of metastatic neuroblastoma in the present study was missed because of marrow necrosis, but aspiration showed small round tumor cells, rosettes and neuropil material with suppression of hematopoiesis. This was also observed by ***Pampa ch Toi et al***.[5]who encountered a single case of metastatic carcinoma in BMA where BMB shows only infarction. This indicates the importance of both the procedures in metastatic carcinomas and shows they are complementary to each other.

We also encountered a single case of tuberculous granuloma which was later confirmed by ZiehlNeelsen staining for AFB in BMB specimen. ***P. Ch. Toi et al.***[5]observed that BMB along with good imprint alone is sufficient in diagnosis of granulomas.

The present study did not show a positive correlation between BMA and BMB in diagnosis of nutritional anemia because aspirate is more useful in diagnosis of macro/ micronormoblasts. A study done by ***P. ch Toi et al***.[5] shows that megaloblasts look like leukemic blast in BMB. Also grading of iron stores are better appreciated and interpreted in BMA than biopsy as decalcification leeches out the iron. Also in cases of reactive marrow, infections like malaria we found that BMA is more helpful than biopsy. A study done by ***Sabharwal et al.***[17] shows that plastic embedding and semi thin sections of undecalcified bone marrow, can be expected to improve the cytological details of tissue obtained by biopsy.

While there are many studies showing the importance of BMB, very few studies in the literature compared the efficacyand importance of BMI in diagnosis of hematological disorders. In the present study we found a **91.20%** positive correlation between BMI and BMB which was quite higher when compared with other studies. (TABLE 3)The percent of positive correlation in case of imprint is also higher when compared with BMA, thus indicating that a well smeared and well stained imprint smears increase the sensitivity in diagnosis of hematological disorders

Conclusion

To, conclude, BMA and BMB along with imprint cytology are complementary to each other. Better results are obtained when both the procedures are performed simultaneously as BMA gives better morphology of the cells and BMB gives a good picture regarding the pattern of distribution of cells, and cellularity of marrow. BMB remains the gold

TABLE 3: POSITIVE CORRELATION OF BMA AND BMI WITH BMB IN VARIOUS STUDIES

OTHER STUDIES	NO. OF CASES	POSITIVE CORRELATION WITH BMB	
		BMA (%)	BMI (%)
P. Ch Toi et al (2010)	160	61.25%	----
S Chandra (2011)	565	78%	84.3%
Vidisha et al (2013)	460	94%	----
V. Tilak et al (2013)	182	----	87.3%
Niranjan et al (2015)	140	75.8%	---
Present study (2013-15)	148	72.04%	91.20%

standard for diagnosing cases when BMA is dry tap, and in cases like hypoplastic/ aplastic anemia and granulomatous inflammation. But, assessment of iron status by Perl's stain is better in BMA than biopsy. A meticulously prepared BMI provides a better information regarding cellularity of marrow than BMA. Also metastatic tumors can be quickly diagnosed in a well prepared imprint smears and unnecessary delay caused by decalcification and processing of BMB specimens in histopathological laboratories can be avoided. Both BMA material and biopsy material can be used for higher studies according to the specific disease, if needed.

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Competing Interest
NIL

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NIL

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