Journal of Pathology of Nepal (2015) Vol. **5**, 739 - 746

Journal of
PATHOLOGY
of Nepal

www.acpnepal.com

Original Article

Bone marrow touch imprint smears as an adjunct to bone marrow aspiration smears in hematological disorders

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Keywords:

Bone marrow;
Imprint;
Myeloid Neoplasms;

ABSTRACT

Background: Morphological examination of the marrow requires a combination of a properly prepared bone marrow aspirate smear, a trephine biopsy section and an imprint of core biopsy. Some conditions often result in a dry tap and are best studied by marrow biopsy. The major drawbacks of biopsy sections are their thickness, precluding fine morphologic detail. The objective of this study was to compare the diagnostic accuracy, cellularity and cytomorphology from bone marrow biopsy core imprint smears with bone marrow aspiration smears.

Materials and Methods: Imprint smears were prepared from 138 cases subjected to bone marrow examination. The bone marrow aspiration, imprint smears and bone marrow biopsy sections were examined and were categorized into five different groups on cytomorphological basis: Non-malignant alterations and normal marrow, Myeloid neoplasms, Plasma cell myeloma, myelo-infiltrative disease and absence of residual disease and further delineated into specific entities wherever necessary.

Results: Out of 138 cases, non-malignant alterations and normal marrow was the largest subgroup (N=87, 63%), followed by myeloid neoplasms (N=26, 18.5%), Plasma cell myeloma (N=13, 9.4%), myelo-infiltrative disease (N=9, 6.5%) and absence of residual disease (N=3, 2.2%). The diagnostic accuracy of imprint smears was highest (92%) followed by biopsy sections (89.9%) and aspiration smears (87%). Kappa analysis showed strong agreement (>0.8) and p-value was statistically significant (<0.001) while correlating the final diagnosis.

Conclusion: Imprint smear technique is a simple, rapid, inexpensive and reliable procedure. The routine use of imprint smear in the bone marrow examination will serve as an invaluable adjunct to bone marrow aspiration and biopsy.

INTRODUCTION

Bone Marrow (BM) examination is a core diagnostic procedure for the evaluation of patients with both malignant and non-malignant hematological disorders. Morphological examination of the marrow requires a combination of a properly prepared bone marrow aspirate smear, a trephine biopsy section and an imprint prepared from the marrow biopsy core at the same setting.[1] Although these procedures have been widely used, a comparative study of these three procedures, to our knowledge has not yet been carried out in our set-up. Smears of aspirated marrow are ideal for the study of cytological detail of hematopoietic cells and

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Table 1: Frequency of different diagnosis grouped under non-malignant alterations and normal marrow

Diagnosis	BMA	BMI	BMB	Total cases
Megaloblastic anemia	32	36	38	36
NMS	11	15	13	14
Mixed erythropoiesis	14	12	10	14
Hypocellular marrow	12	9	8	8
No myelo-infiltrative disease	5	6	6	6
Decreased megakaryocytes	0	0	2	2
Increased megakaryocytes	2	2	2	2
Increased eosinophilic precursors	2	2	2	2
Micronormoblastic erythropoiesis	1	2	1	1
Granuloma	0	0	1	1
Visceral Leishmaniasis	1	1	0	1
Hypercellular marrow	2	2	0	0
Total	82	87	83	87

Table 2: Diagnostic accuracy of different diseases grouped under myeloid neoplasms by the three techniques.

Diagnosis	BMA Number of cases	BMI Number of cases	BMB Number of cases	Total Cases
Acute leukemia	9	11	14	10
AML	4	3	0	4
CML-CP	5	6	6	5
CML-AP	2	1	0	2
Remission cannot be ruled out	1	1	1	1
MDS	1	1	1	1
AML not in remission	3	3	2	3
Absence of residual disease	1	1	1	1
Total	26	27	25	27

Table 3: Final impression on bone marrow study

Final Impression sub-groups	No of Cases (%)
Non-malignant alterations and normal marrow	87(63.1)
Myeloid neoplasms	26(18.8)
Plasma cell myeloma	13(9.4)
Myeloinfiltrative diseases	9(6.5)
Absence of residual diseases	3(2.2)
Total	138(100)

suitable for cytochemical studies. However, needle biopsy permits histologic evaluation of marrow architecture and quantification of various cell populations, which cannot be accomplished by the aspiration smears alone.[1] Conditions such as aplastic anemia, myelofibrosis, and myelophthisic lesions often result in a dry tap on repeated bone marrow aspirations and are best studied by marrow biopsy. The major drawbacks of biopsy sections are their thickness, which may preclude study of fine morphologic detail. Individual cell morphology and differential counts of cellular elements can be obtained more accurately from particle smears and biopsy touch imprints.[1] Moreover imprints may prove better in evaluating cell morphology and rapidly assessing cellularity. A specific diagnostic role for Bone Marrow Imprints (BMI) has not been adequately explored and a large population-based study has rarely been reported.[2] This study was conducted to evaluate the role of BMAs and touch imprints of BMB to optimize diagnostic utility of BM study, which would be important in better patient management.

MATERIALS AND METHODS

This was a prospective, hospital- based cross sectional study done on 138 cases. Bone marrow aspiration and biopsy were done at the Department of Pathology, Bir Hospital whenever clinically indicated over a period of one year (1st November 2012 to 31st October 2013), under local anaesthesia after obtaining informed consent. All patients having hematological disorders subjected to bone marrow aspiration and biopsy were included in the study. Patients who refused to take part in study were excluded. Patient's clinical details were documented.

Bone marrow aspirations of all the patients were performed taking universal precautions and standard procedure. At least seven smears were made immediately and 3 smears were stained with Giemsa's stain after air drying.[3] Subsequently, the biopsy was performed following which the bone marrow trephine biopsy imprint was made. The fresh biopsy core was gently rolled as a circle in one direction on the glass microscope slide. If the biopsy core was bloody, sterilized gauze was used to absorb excess liquid to obtain useful bone marrow trephine biopsy core imprints.[1] Then the BM biopsy core was fixed in Bouin's fluid and processed for paraffin-wax embedding.[4] Bone marrow trephine biopsy core imprint slide was dried and subjected to fixation and staining procedure along with the aspiration slides. Prepared cytology slides and biopsy slides were examined under the light microscope.

The bone marrow smears were examined as mentioned below. Number of megakaryocytes was expressed as number in 10x low power fields. The cellularity was graded into seven grades: severe hypocellular, moderate hypocellular, mild hypocellular, normo-cellular, mild hypercellular, moderate hypercellular and severe hypercellular.[1] With BM trephine biopsy imprints and smears, 100 oil-immersion fields of nucleated cells were screened, on BM smears near the tail, and the seven grades were designated according to the average number of nucleated cells per oil-immersion field (<5, 5–14, 15–24,25–50, 51–70, 71–90 and >90 respectively). Ten fields were assessed and average was considered. With BM trephine sections, the seven grades were divided according to the percentage of hematopoietic tissue (<10, 11–25,26–40, 41–64, 65–77, 78–90 and >90%). Ten fields were assessed and average was considered. As the trephine section is the best method for evaluating the BM cellularity, cellularity on sections was taken as the

Table 1: Frequency of different diagnosis grouped under non-malignant alterations and normal marrow

Cellularity in BMA total nucleated cell count in oil immersion (100 x)	Cellularity in BMI in total nucleated cell count in oil immersion (100x)									
		Inadequate	< 5 cells severe hypo-cellular	5- 14 cells moderate hypocellular	15- 24 cells mild hypocel-lular	25- 50 cells normocel-lular	51 - 70 cells mild hyper-cellular	71 - 90 cells moderate hypercellular	> 90 cells marked hy-percellular	Total
	Dry Tap	0	0	9.1% (1)	9.1% (1)	54.5% (6)	27.3% (3)	0	0	100% (11)
	< 5 cells severe hypocel-lula r	0	50.0% (1)	0	50.0% (1)	0	0	0	0	100% (2)
	5-14 cells moderate hypocellula r	0	16.7% (3)	44.4% (8)	5.6% (1)	27.8% (5)	5.6% (1)	0	0	100% (18)
	15-24 cells mild hypocel-lula r	0	0	7.7% (1)	38.5% (5)	38.5% (5)	15.4% (2)	0	0	100% (18)
	25-50 cells normocellular	2.1% (1)	2.1% (1)	0	6.4% (3)	70.2% (33)	17.0% (8)	2.1% (1)	0	100% (47)
	51- 70 cells mild hypercel-lular	3.3% (1)	0	0	3.3% (1)	13.3% (4)	66.7% (20)	13.3% (4)	0	100% (30)
	71 - 90 cells moderate hypercellular	0	0	0	0	0	11.1% (1)	88.9% (8)	0	100% (9)
	> 90 cells marked hyper-cellula	0	0	0	0	0	0	12.5% (7)	87.5% (7)	100% (8)
	Total	**1.4% (2)**	**3.6% (5)**	**7.2% (10)**	**8.7% (12)**	**38.4% (53)**	**25.4% (35)**	**10.1% (14)**	**5.1% (7)**	**100% (138)**

Kappa : 0.483 : moderate agreement, p-value < 0.001

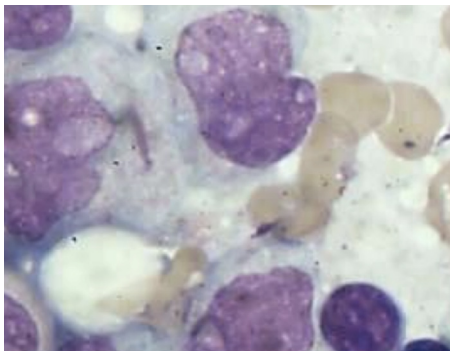

Figure 1: Bone marrow imprint smear showing myeloblasts with Auer rod in AML (Giemsa stain, X400).

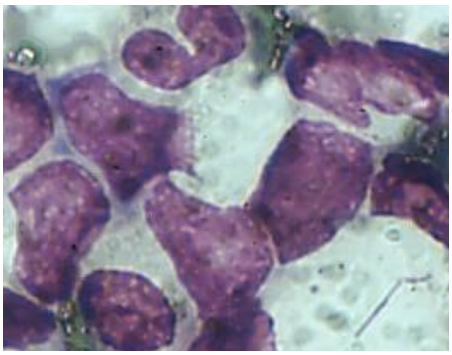

Figure 2: Bone marrow imprint smear showing atypical lymphoid cells infiltration (Giemsa stain, X400).

reference in this study, and we focused on comparison of cellularity between BM trephine biopsy core imprints and smears. Cellularity from the bone marrow trephine sections were also calculated from the standard formula : Marrow cellularity = (100 – patient age) % ± 20%.[5] Diagnosis was made separately in both the aspiration and imprint smears and also on trephine sections.

RESULTS

There were total 138 cases included in this study. The mean age of the patients was 40.75 with 29 patients in the age group of 21- 30 years and 25 patients in 41-50 years. There were more males (58%) than females (42%) with a male to female ratio of 1.37. There were 11 dry tap cases and two inadequate imprint smears. All the dry tap cases were diagnosed in the bone marrow imprint smears. Diagnostic accuracy of bone marrow aspiration smears was 87.0 % and

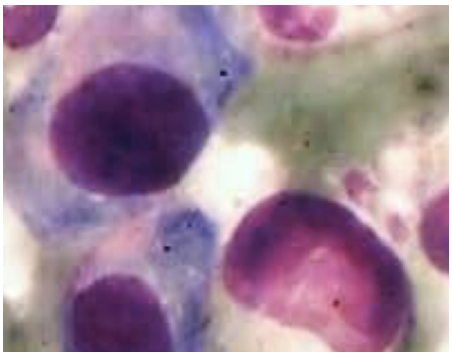

Figure 3 Bone marrow imprint smear showing plasma cells (flame cells)(Giemsa stain, X400).

that of bone marrow imprint smears was 92.0%. The final impression (on cytomorphological basis) was divided into five sub-groups:

a)Non-malignant alterations and normal marrow,

b)Myeloid neoplasms,

c)Plasma cell myeloma

d)Myeloinfiltrative diseases, and

e)Absence of residual disease

Out of 11 dry tap cases of BMA diagnosed on BMI smears, 4 were cases of non-malignant alterations and normal marrow, 3 were myelo-infiltrative diseases, 2 were cases of plasma cell myeloma and 1 each was of absence of residual disease and myeloid neoplasms. Two BMI smears were inadequate. Both of them were reported as non-malignant alterations and normal marrow in BMA smears.

Non-malignant alterations and normal marrow

This group included even the cases with normal marrow study. The most common diagnosis was megaloblastic anemia. Six cases grouped under no myelo-infiltrative disease were cases of lymphoma subjected to BM evaluation for staging and after chemotherapy(Table. 1).

Myeloid Neoplasms

Out of 26 total cases grouped under myeloid neoplasms 10 cases were of acute leukemia, 7 of chronic myelogenous leukemia (CML), 4 of acute myeloid leukemia (AML) and 1 of myelodysplastic syndrome (MDS). In one of the case remission of AML could not be ruled out on cytomorphological basis and 3 follow up cases of AML still had residual disease. Only one case included in this study of AML was in remission. (Table 2)

Myelo-infiltrative diseases

There were 15 cases of lymphoma: 3 of Hodgkin lymphoma (HL) and 12 of Non-Hodgkin lymphoma (NHL). One case of HL was post chemotherapy. All the cases of HL and three cases of NHL were negative for BM infiltration. Only the cases with positive atypical lymphoid cells were studied for cytomorphology and pattern of arrangement in the BMB sections.

DISCUSSION

Out of the 138 cases, 11 cases yielded dry tap in bone marrow aspiration. Those 11 cases were studied only on imprint smears and trephine biopsy sections. Two of the BMI smears included in this study were inadequate and evaluation was done only with aspiration smears and biopsy sections. In the study by Donald P et al. involving, 108 cases, there were 12 dry taps on aspiration and 7 samples with inadequate touch preparations. They have explained that applying too great pressure during preparation of imprint smears cause cell breakage and makes interpretation difficult.[6]

A total of 138 cases were grouped into 24 different disease processes after the cytomorphological evaluation of bone marrow. The most common group was of megaloblastic anemia which was seen in 36 cases (26.1%). Das Makheja S et al observed similar finding. Of 62 cases of pancytopenias he observed that the most common diagnosis was megaloblastic anemia which was present in 26 cases(41.9%).[7] However, in the study by Kibria SG et al. for evaluation of various hematological disorders, the most common diagnosis was AML which comprised 49.67% of cases.[8] In our study; acute leukemia accounted for 7.2%; out of which AML for 2.9%. There were 14 cases (10.1%) where there was no significant alteration in marrow architecture and its hematopoietic elements, and were reported as normal marrow study. In the study done by Chandra S et al., 80 of the 565 cases were reported as normal marrow study in biopsy sections, 75 in imprint cytology and 58 in aspiration smears. The next most common category after normal marrow and megaloblastic anaemia was of mixed erythropoiesis comprising of 10.1% of cases. This finding was similar to that of the study done by Kibria SG et al. where incidence of combined anemia followed AML .There were two cases (1.4%) reported as presence of increased eosinophilic precursors. They were cases of peripheral blood eosinophilia and eosiniphilicleukaemia was ruled out in them.

When the diagnostic accuracy of the three different techniques were compared, the highest accuracy was achieved from the BMI smears (92%) followed by BMB sections (89.9%) and BMA smears(87%). The lower diagnostic accuracy of BMA smears may be attributed to the eleven cases resulting in dry tap. In the study done by Chandra S et al. the overall diagnostic accuracy was highest for the biopsy sections (99.2%) followed by imprint smears (83.7%). The diagnostic accuracy of imprint smears was significantly higher than the BMA smears (77.5%) in their study.

The twenty four various diagnoses in our study were stratified into 5 groups (Table 3). Non-malignant alterations and normal marrow study was the largest group accounting for 63% (87 of the cases). This finding is similar to that from the study done by Gong X et al. in a large population of people (3781 cases) where the most common diagnosis was non-malignant hematologic diseases including the normal marrow study.[1] The second largest group was of myeloid neoplasms comprising of 26 cases (18.5%). This group included a single case of MDS as wel; hence it was named as myeloid neoplasms instead of haemotologic malignancies.

Myeloid tumors was also the second largest group in the study done by Gong X et al. There were 3 cases (2.2%) grouped under absence of residual disease. This group included follow-up cases of leukemia (1 case) and plasma cell myeloma (2 cases). All the three cases were subjected to BM evaluation after completion of chemotherapy. Out of 15 cases of lymphoma subjected to BM evaluation, 9 cases were positive for BM infiltration, which were grouped under myelo-infiltrative diseases. There were 13 cases (9.4%) of PCM which were kept separately.

Non-malignant alterations and normal marrow

This group constituted the largest group of cases in our study with 87 cases (63%). There were 3 cases of mixed erythropoiesis in both the BMA and BMI smears, which were diagnosed as only megaloblastic maturation in BMB sections. As cytomorphology is better appreciated in the aspiration and imprint smears,[6] we considered their finding as final. A case of megaloblastic anemia, diagnosed in BMA and BMI smears, yielded scanty hemopoeitic tissue and bony trabeculae and was deemed inadequate for assessment in the BMB sections. Two cases of megaloblastic anemia yielded dry taps on aspiration. Two cases diagnosed as megaloblastic anemia in BMI and BMB sections were missed in BMA smears and were diagnosed as hypocellular marrow. In all the cases of megaloblastic anemia, serum vitamin B12 and folic acid assays were recommended.

Taking all the three diagnostic modalities into consideration, 14 cases (17.24%) were reported as normal marrow study . Out of the 15 cases that were diagnosed as normal marrow in BMI smears, a case was diagnosed as PCM and another case as myelo-infiltrative disease from BMA and BMB sections studies. There were two cases diagnosed as hypercellular marrow in both BMA and BMI smears but both of them had normal cellularity in the BMB sections. A case of normal marrow yielded dry tap on aspiration. One case reported as normal marrow in both BMI and biopsy sections was reported as hypocellular marrow in the BMA smears.There were only 11 cases reported as normal marrow study in BMA smears. One case reported as normal marrow in BMA smears was inadequate in the BMI smears and was diagnosed as granulomatous lesion in trephine biopsy sections.

There were 14 cases of mixed erythropoiesis. All were correctly identified as having mixed erythropoiesis in the BMA smears. The two cases in BMI smears showed discrepant findings from aspiration smears and were deemed inadequate and micronormoblastic erythroid maturation. In trephine biopsy sections, there were 4 discrepant cases. Three cases were reported as megaloblastic maturation and one case as hypocellular.

There were 10 cases having mixed megaloblastic and micronormoblastic maturation and 2 cases having mixed normoblastic and megaloblastic maturation in the BMI smears. There were 11 cases having mixed megaloblastic and micronormoblastic maturation and 3 cases having mixed normoblastic and megaloblastic maturation in the BMA smears. BMI smears were inferior to BMA smears in identifying the mixed erythropoiesis cytomorphology in our study.

There were 12 cases reported as hypocellular marrow in BMA smears. Out of these one was reported as decreased megakaryocytes, another case as a normal marrow and two cases as megaloblastic anemia in biopsy sections. There were 9 cases reported as hypocellular marrow in BMI smears. One case showed only decreased megakaryocytes with normal cellularity in the BMB sections. As biopsy sections are taken as the gold standard technique for assessment of cellularity, we too have emphasized on the findings in the trephine sections. In our study rate of detection of hypocellular marrow in imprint smears was higher than that of BMA smears resulting in less number of false positive cases. Our finding in this study is similar to the study done by Gong X et al., where the false positive diagnostic rate of imprints for aplastic anemia (0.29%) was lower than in smears (1.09%). They have concluded that in aplastic anemia patients whose diagnoses were highly corroborated to cellularity of the BM, the diagnostic impact of BM imprints was better than smears.[1]

Six cases grouped under no myelo-infiltrative diseases (N=6/15; 40%) were cases of lymphoma subjected to BM evaluation for staging. There were three cases of HL, among which two were subjected to bone marrow examination for staging and the remaining one case was subjected to bone marrow after chemotherapy to find out the cause of persistent cytopenia. All the three cases were negative for myelo-infiltrative diseases. Out of 9 cases of NHL, 3 cases subjected to bone marrow evaluation for staging were negative for myelo-infiltrative diseases. There was a case which yielded dry tap in aspiration which had no myelo-infiltrative disease. The diagnostic accuracy of BMI smears was 100% in this group as par with BMB sections. In the study done by Moid et al., the infiltration of marrow by Hodgkin's lymphoma was present in 30% of the total cases.[9] Due to paucity of cases with the aforementioned disease in our study, rate of infiltration of marrow could not be determined.

Two cases showed decrease in the number of megakaryocytes in our study. Both the cases had normal bone marrow except for decreased megakaryocytes number.

Two cases showed increased megakaryopoiesis. One case was subjected to bone marrow evaluation for pyrexia of unknown origin. The other case was suspected case of ITP with thrombocytopenia. Increased megakaryopoiesis gives a clue of the peripheral destruction of platelets and likely diagnosis of idiopathic thrombocytopenic purpura (ITP).[10]

There were two cases (2.3%) reported as increased number of eosinophilic precursors. Both the cases were subjected to bone marrow study for persistent eosinophilia of duration greater than six months. As the percentages of myeloblasts were normal, we reported the cases as increased eosinophilic precursors. One of these cases was a patient with pulmonary tuberculosis under anti-tubercular drug therapy (ATT). ATT is a well-known cause for reactive eosinophilia,11 which explains the probable cause of increased eosinophilic precursors in this patient.

There was only one case of micronormoblastic erythropoiesis in our study. In imprint smears two cases were reported as micronormblastic erythropoiesis. A case of mixed erythropoiesis was reported as micronormoblastic maturation in the imprint smear.

There was a single case each of visceral leishmaniasis and granulomatous disease in the study. We were able to identify Leishman-Donovan bodies even in the BMI smears. In the single case of granuloma, both BMI smear and aspiration studies were unable to reveal granulomas. Many of the studies done in the past have shown that due to focal involvement of marrow by the disease process, granulomas are often missed in the BMA smears and require BMB sections.[2, 12]

Two cases were reported as hypercellular marrow both in the aspiration and imprint smears. Examination of biopsy sections of those cases revealed normal cellularity and thus both of them were regarded as normal marrow in the final impression.

Myeloid neoplasms

Out of 26 total cases grouped under myeloid neoplasms category, ten cases were of acute leukemia, 7 cases of CML, 4 cases of AML and one of MDS. In one case, remission of AML could not be ruled out on cytomorphological basis and 3 follow -up cases of AML still had residual disease. Only one case included in this study had full remission. Using all these three modalities for bone marrow study, we were able to diagnose ten cases with acute leukemia. The presence of Auer rod is needed to delineate acute leukaemia as AML .Except for a single dry tap case, all the cases were correctly diagnosed as acute leukemia and AML in the BMA smears. In one case of AML, Auer rods were not seen in the BMI smear, so it was categorized as acute leukemia from the imprint smears study. None of the cases of AML showed Auer rods in the myeloblasts in the BMB sections, hence all of them were diagnosed as acute leukemia in the trephine sections. Marrow biopsy sections do not help in the identification of Auer rods and thus categorization of acute leukaemia into AML is not possible.BMA smears had highest diagnostic accuracy in diagnosing acute leukemia and acute myelogenic leukemia. However, imprint smears were found to be useful in the case of dry tap on aspiration. Even in the study done by Gong X et al., diagnostic accuracy of AML was highest in BMA smears (90.9%) followed by that obtained from imprint smears (89%).

In one case of AML, Auer rods were not seen in the imprint smears. Gong X et al. in their study have further explained that because cells on imprints are not pushed as in smears, cells are crowded together in hypercellular regions and cytomorphological analyses are affected(especially the cytoplasm and granules). [1]This may be the reason why Auer rod was missed in the single case even in our study.

Less than or equal to 5% blasts in the BM, no Auer rods in myeloblasts, recovery of neutrophils and platelets, and the absence of extramedullary disease constitute the cornerstones for the definition of a hematological complete remission (CR) in patients with AML.[13] In the single case, the blasts population was less than 5%, and there were no Auer rods although hematological parameters reflected otherwise. We therefore advised for the cytogenetic analysis for further assessment of the disease process. Out of five cases of AML subjected to bone marrow after completion of chemotherapy only one had absence of residual disease.

Out of seven cases of CML, five cases were in chronic phase and 2 cases were in accelerated phase. Chronic myeloid leukemia is said to be in accelerated phase if the blast count in marrow smears range between 5-19%.[14] In one case diagnosed as CML-AP in aspiration smears, imprint smear showed blast count less than 5%. As discussed by Gong X et al. in their study because of the hypercellular smears the cytomorphological details of blasts may have been missed since cells are not evenly spread in imprint smears.[1] Both the cases of CML-AP were missed in trephine sections.

There was a single case of MDS in our study. The clinical presentation was of refractory anemia lasting for more than six months. On both the aspiration smears and the imprint smears, dysplastic features(in greater than 10% of the cells) of erythroid precursors in the form of megaloblastoid changes, multi-nucleation, budding and bridging, dysplastic features(in greater than 10% of the cells) of myeloid precursors in the form of hypogranulation, Pseudo-PelgerHuet cells and hypolobated and hypogranular megakaryocytes were seen.[14] Even in the bone marrow biopsy sections hypolobated and hypo-granular megakaryocytes were seen. Considering the clinical presentation and the age of the patient we gave the diagnosis of MDS and advised for cytogenetic analysis for further confirmation.

Plasma cell myeloma

There were total of 15 cases of Plasma cell myeloma, out of which two cases were in remission. Total of six cases had persistent disease even after chemotherapy. There were six cases of newly diagnosed plasma cell myeloma and there was one case where clinical information were highly

suggestive of PCM (lytic rib lesion, plasma cells in the pleural fluid) but we could not clinch the diagnosis of PCM in BM because plasma cells were although increased less than 10%of the total cells. In the repeat BMA of the same case from another site, we could confirm the diagnosis of PCM. This finding agrees the importance of bilateral BM examination in the PCM as advocated by Almeida et al.[15]

Out of 10 cases in the BMA smears with greater than 10% of plasma cells, only one was missed in BMI smear. There were total of 11 cases of plasma cells greater than 10% in the BMI smears, in contrast to 10 cases from BMA smears. The two cases in which PCM was missed in the BMA yielded dry tap on aspiration. This finding again reinforces the utility of BMI in the diagnosis of hematological disorders in case of dry tap during aspiration.[3]

The diagnostic accuracy of plasma cell myeloma was in the following order: BMB section (92.3%), BMI (84.6%) and BMA smears (76.92%) in this study. This finding re-enforce the combined use of BMA and BMB sections for the diagnosis of PCM in accordance with the findings of the study by Stifter S et al.[16]

Myelo-infiltrative diseases

There were total number of 15 cases of lymphoma in this study. Three cases were that of HL and 12 cases were of NHL. Only the cases with positive atypical lymphoid cells (9 out of 12 NHL cases) were studied for cytomorphology and pattern of infiltration in the BMB sections.

In the study done by Zemunik T et al., overall incidence of bone marrow infiltration by low grade NHL was 47% which was much less than in our study. We had 75% (9 out of 12 cases) of NHL with positive bone marrow infiltration. The sample size included in their study was 60 in comparsion to 15 cases in our study.[17]

Assessment of Cellularity

Cellularity was categorized into seven different grades in this study. There were 2(1.45%)severe hypocellular cases,18 (13.04%)moderate hypocellular cases, 13(9.42%) mild hypocellular cases, 47(34.05%) normocellular cases, 30(21.73%) mild hypercellular cases,9(6.52%) moderate hypercellular cases and 8(5.79%) marked hypercellular cases in the BMA smears.Similarly, there were 5(3.6%) severe hypocellular cases, 10(7.2%)moderate hypocellular cases, 12(8.7%) mild hypocellular cases, 53(38.4%) normocellular cases, 35(25.4%) mild hypercellular cases, 14(10.1%) moderate hypercellular cases and 7(5.1%) marked hypercellular cases in the BMI smears. (4)

As depicted by Table 4, the correlation of normocellular, moderate and markedly hypercellular smears between aspiration and imprint smears were stronger than the remaining grades. Kappa analysis showed moderate agreement (kappa: 0.483) between two studies and p-value was also statistically significant in this study. Correlation of cellularity in aspiration and imprint smears were better than correlation of cellularity of either BMA or BMI smears with BMB sections (fair agreement).

These findings in our study are similar to the findings described by Gong X et al. In their study, no statistical difference was found in grades of cellularity obtained from imprint and trephine samples. Cases categorized as extreme, obvious and slight hypocellularity were fewer in the BM imprint than aspiration smears ($P < 0.05$). In contrast more cases were categorized as slight, obvious and extreme hypercellularity in the imprint smears than in the aspiration smears ($P < 0.05$). We used the criteria used by Gong X et al. for grading the cellularity.[1]

Aboul NR et al. in their study had found no diagnostic difference in the differential counts from touch imprints and aspirate smears of normocellular bone marrow. Our study also showed 70.2% correlation between these two smears in cases with normocellular marrow. Although they found some difference between the differential counts in certain cases of diseased bone marrow, the touch imprint proved to be a reliable diagnostic tool for determining the cellular composition of normal bone marrow and more reliable for the diagnosis of bone marrow involved by a neoplastic hematologic diseases.[18] In our study too, there was good correlation between cellularity of the aspiration smears and imprint smears in the normal marrow as well as the marrow involved by various non-neoplastic and neoplastic hematological disorders.

CONSLUSION

Imprint smear is a simple, rapid and reliable technique, which gives excellent cytomorphological details. Imprint smears has combined features of both the aspiration smears and trephine sections. Cellularity and cytomorphology obtained from imprint smears is equally informative to that obtained from aspiration smears. Imprints are better than bone marrow trephine sections for cytomorphological analyses without immunophenotype and the diagnostic accuracy of imprints for cytomorphology based diseases is better than trephine sections. Thus, imprint cytology must be used as an adjunct to bone marrow aspiration and biopsy.

This study recommends the routine preparation and study of bone marrow trephine core imprint smears in evaluation of bone marrow diseases.

REFERENCES

1. Gong X LX, Wu X,Xu R,Tang Q,Xu G,Wang L,Zhang X,Zhao X. Role of bone marrow imprints in haematological diagnosis: a detailed study of 3781 cases. Cytopathology. 2012 Apr;23:86-95. Crossref

2. Toi P, Varghese RG, Rai R. Comparative evaluation of simultaneous bone marrow aspiration and bone marrow biopsy: an institutional experience. Indian J Hematol Blood Transfus. 2010 Jun;26:41-4. Crossref

3. James LP, Stass SA, HR. S. Value of imprint preparations of bone marrow biopsies in hematologic diagnosis. Cancer. 1980;46:173-7. Crossref

4. Bancroft JD GM. Theory and practice of histological techniques. sixth ed: Churchill Livingstone Elsevier; USA; 2008. 725p.

5. Peter A. Humphrey, Louis P. Dehner, John D. Pfeifer. The Washington Manual of Surgical Pathology: wolters Kluwer; USA; 2008. 784pp.

6. Donald P, G. C. Comparative evaluation of bone marrow aspirate particle smears, biopsy imprints, and biopsy sections. Am J Hematol. 1986;22:381-9. Crossref

7. Das Makheja K, Kumar Maheshwari B, Arain S, Kumar S, Kumari S, Vikash. The common causes leading to pancytopenia in patients presenting to tertiary care hospital. Pak J Med Sci. 2013;29:1108-11. Crossref

8. Kibria SG IM, Chowdhury ASMJ, Ali MY, Haque MR, Mustanzid SM, Ali SY. Prevalence Of Hematological Disorder: A Bone Marrow Study Of 177 Cases In A Private Hospital At Faridpur. Faridpur Med Coll J. 2010;5:11-3.

9. Moid F, DePalma L. Comparison of relative value of bone marrow aspirates and bone marrow trephine biopsies in the diagnosis of solid tumor metastasis and Hodgkin lymphoma: institutional experience and literature review. Arch Pathol Lab Med. 2005;129:497-501. Crossref

10. Greer JPJ, Rodgers GM. Wintrobe's Clinical Hematology: Lippincott Wiliams and Wilkins; 2009. 2312p.

11. Mckenzie SB. Textbook of Hematology. second edition ed: Williams and Wilkins; Baltimore;1996.1876p.

12. Chandra S CH. Comparison of bone marrow aspirate cytology, touch imprint cytology and trephine biopsy for bone marrow evaluation. Hematology reports 2011;3:65-8. Crossref

13. de Greef GE, van Putten WL, Boogaerts M, Huijgens PC, Verdonck LF, Vellenga E, et al. Criteria for defining a complete remission in acute myeloid leukaemia revisited. An analysis of patients treated in HOVON-SAKK co-operative group studies. Br J Haematol. 2005;128:184-91. Crossref

14. Swerdlow S H CE, Harris N L et al. WHO classification of tumors of hematopoietic and lymphoid tissues. 4th edition ed. F T. Bosman ESJea, editor: IARC 69008,lyon, France; 2008.

15. Almeida J, Garcia-Marcos MA, Vallejo C, Flores MT, Caballero MD, San Miguel JF, et al. Results of a series of 104 consecutive bilateral bone marrow biopsy specimens in lymphoproliferative disorders. Sangre (Barc). 1995;40:365-8.

16. Stifter S, Babarovic E, Valkovic T, Seili-Bekafigo I, Stemberger C, Nacinovic A, et al. Combined evaluation of bone marrow aspirate and biopsy is superior in the prognosis of multiple myeloma. Diagn Pathol. 2010;5:30. Crossref

17. Zemunik T, Vuckovic J, Marinkovic M, Forempoher G. Bone marrow involvement and the prognosis of low grade non-Hodgkin's lymphoma. Croat Med J. 1998;39:419-21. Crossref

18. Aboul NR, Estey EH, Kantarjian HM, Freireich EJ, Andreeff M, Johnson BJ, et al. Comparison of touch imprints with aspirate smears for evaluating bone marrow specimens. Am J Clin Pathol. 1999: 111;753-8.