Original Article

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Role of Immunohistochemistry in Trephine Biopsies of Bone Marrow: A 5-year Retrospective Study from A Tertiary Care Hospital

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ABSTRACT

Background: Bone Marrow Biospy (BMB) often needs ancillary tests like Immunohistochemistry (IHC) to confirm the morphological diagnosis, to categorize malignant conditions.

Methods: A retrospective study was done on BMB with IHC done on the sections are reviewed and analysed correlating the clinical, Bone marrow aspirate(BMA) and BMB interpretations.

Result: A 5 year study on 934 BMB required IHC as an adjunct on 16.2% of the biopsies. It was done on 10.6% of cases. The distribution of cases was 43% of Acute leukemia(AL), 19% multiple myeloma, 8& lymphomas, 9% metastasis. 20 % of other cases which included chronic lymphocytic leukemia(CLL), myelodysplastic syndrome (MDS), megaloblastic anemias, infective conditions and reactive marrows.

Conclusion: IHC is a reliable method to confirm and categorize AL, to differentiate reactive and neoplastic plasmacytosis, to confirm, categorize and stage lymphomas, to detect metastasis.

Keywords: *Bone Marrow, Immunohistochemistry, Leukemia, Metastasis, Trephine Biopsy*

Introduction

Bone marrow interspersed within the intertrabecular spaces of the medullary cavity in the bone, has complex anatomy and physiology which are valuable to support almost all the systemic functions. Its involvement in diseases varies either from within as primary marrow diseases or as reflected changes of systemic diseases.

Bone marrow studies are indicated in various haematological and non-haematological disorders. Bone marrow trephine biopsy (BMB) had been standardized in 1943 after many trials and studies since it was first performed by Mosler.[1,2] It had been suggested that bone marrow aspirates (BMA) and BMB should be assessed by a competent person.[3] The need for histochemical and immunohistochemical stains depend on the clinical circumstances and the preliminary findings. Indications for immunohistochemistry (IHC) on BMB depend on the morphology and the panels are often classified as primary panel and secondary panel. In this era of immunophenotyping, IHC is almost always done for lymphoma diagnosis and categorization in lymph nodes. However International Council for Standardization of in Haematology (ICSH) had set guidelines for the standardization of bone marrow IHC.[4]

The present study is aimed to discuss the diagnostic utility of IHC in BMB.

Materials and Methods

This is a retrospective study done on BMB reported in the department of pathology for a period of 5 years (Jan 2011-Dec 2015). The bone marrow registers were reviewed and all bone marrow biopsies for which IHC was requested were selected. All the details regarding clinical scenario, relevant laboratory parameters and BMA reports available in the registers were tabulated.

The IHC based diagnoses were grouped into acute leukemias (AL), multiple myeloma (MM), lymphoma, metastatic deposits and others. The demography, clinical details, peripheral blood findings, BMA morphology and BMB were studied. Standard descriptive statistics were used.

Result

A total of 934 BMB were received during the study period of 5 years (2011-2015). The pathologists required IHC as an adjunct to morphology in 151 (16.2%) cases. IHC was done on 99 (10.6%) biopsies and not done on 52 (5.6%) cases. IHC was done with the BioGenex Xmatrx automated staining with rabbit and mouse monoclonal antibodies CD45, MPO, CD20, CD3, CD5, CD10, Tdt, CD34, CD117, CD68, CD138, kappa, lambda, fascin, BCL2, cyclin D1, PAX5, CD10, ALK and EMA as primary

antibodies. The chromogen used was 3,3 diaminobenzidine (DAB). The age of the patients ranged from less than 1 year to 83 years with a male: female ratio of 1.5:1. Table 1 shows the distribution of the cases with clinical diagnosis.

AL was diagnosed on 43% of BMB of which 69.7% were clinically suspected as inflammatory conditions. The BMA was inadequate in 48.8% cases and 6.97% were false negative.

Multiple myeloma was diagnosed on 19% of the biopsies by IHC (Fig 1A, 1B). 21% were not suspected and 26.3% BMA were inadequate. 10.5% BMA were reported as false negative.

Lymphoma infiltration diagnosed on morphology was confirmed and categorized on 8% of biopsies by IHC. Among them only 25% were received for staging. Other 75% of BMB showed lymphoma infiltration which was not suspected clinically. 62.5% BMA were inadequate and false negative report was provided in 12.5% cases.

Metastasis was identified in 9% of cases of which 33.3% cases were from known cases of primary solid tumors (Fig 2B,2B). Malignant solid tumors were not suspected in 66.7% cases. BMA was inadequate in 33.3% of cases.

The other 20% of cases included leukemic conditions (chronic myeloid leukemia-1, chronic lymphocytic leukemia-2, myelodysplastic syndrome (MDS-4), 15% anemia cases (megaloblastic anemia -2, aplastic anemia-1), inflammatory conditions (15%) and reactive marrows(33.3%).The reactive marrows were suspected to be MM, MDS and ALs. IHC was contributory to the diagnosis in 46% of the cases.

Discussion

BMA and BMB play an important role in the diagnosis of various haematological and non-hematological diseases. BMA are good samples for morphologic assessment, cell count, flowcytometry (FCM) and genetic workup. Sampling errors are common due to disease conditions or technical default. IHC studies are possible on BMB which aid in confirming or detecting various disease conditions. In this study done on the IHC work up of BMB, AL was confirmed and categorized in 43% of cases. FCM on BMA and peripheral blood if the blast count is high is

Table 1: Clinicopathological correlation by IHC.

Category By IHC	CLINICAL DIAGNOSIS		No. of cases	BMB & IHC	
ACUTE LEUKEMIA (n=43)	ACUTE LEUKEMIA ANEMIA SUSPECTED MALIGANACY PANCYTOPENIA MDS RELAPSE INFLAMMATORY/INFECTIOUS OTHERS		13 8 8 6 4 2 1 1	AML 6 4 3 3 4 0 0 1	ALL 7 4 5 3 0 2 1 0
MULTIPLE MYELOMA (n=19)	Multiple myeloma Lymphoma MDS Anemia		15 1 2 1	Kappa 9	Lambda 10
LYMPHOMA (n=8)	Lymphoma Pancytopenia Inflammatory/infectious MDS Others		2 3 1 1 1	HL 5	NHL 3
METASTATIC BONE DISEASE (n=9)	KNOWN PRIMARY	Prostatic adenocarcinoma Retinoblastoma	2 1	2 1 Prostatic carcinoma Adenocarcinoma lung Metastatic carcinoma Rhabdomyosarcoma GIT metastasis	
	UNKNOWN PRIMARY	MDS Multiple Myeloma Anemia PNET Gastric ulcer	1 1 2 1 1		
Others(n=20)					

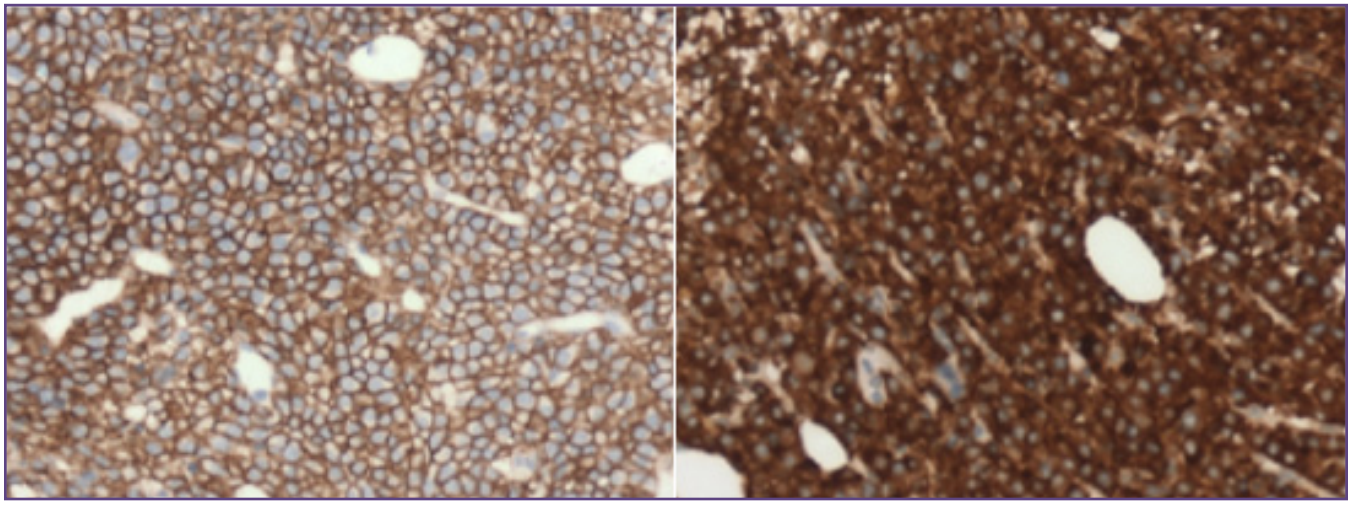

Fig. 1: A Photomicrograph shows sheets of plasma cells positive for CD138, IHC (20X); Fig 1B Photomicrograph shows sheets of plasma cells positive for Lambda light chain, IHC (20X).

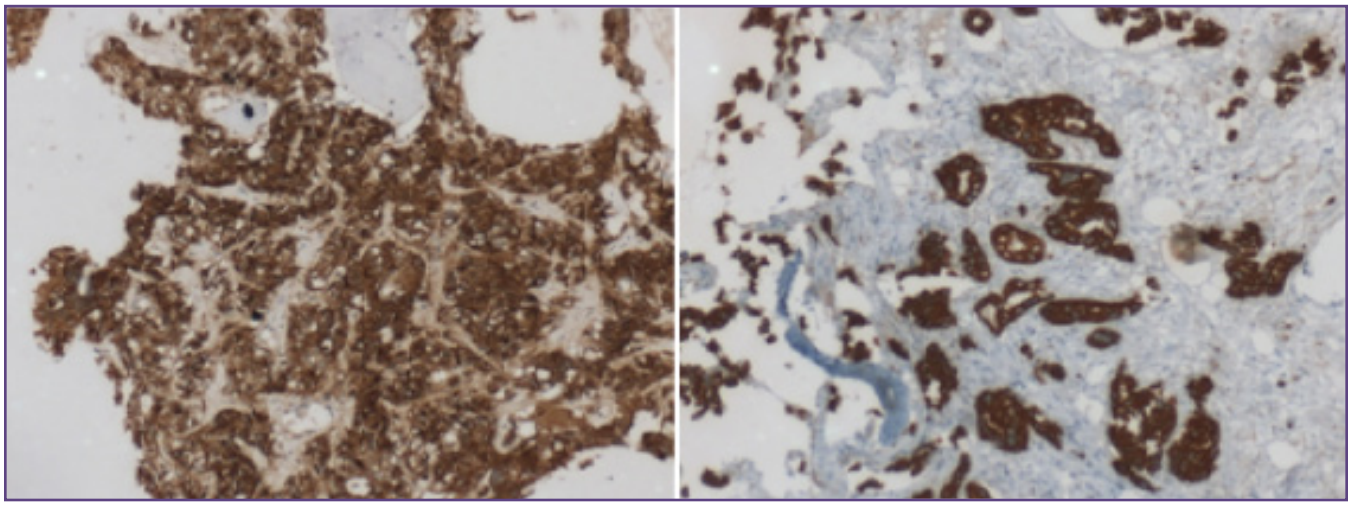

Fig. 2: A Photomicrograph shows glands & sheets of tumor cells positive for PSA, IHC (20X); Fig 2B Photomicrograph shows tumor cells arranged in glands positive for CK7, IHC (20X).

the method of choice for immunophenotyping in AL.[5] However, this was not possible when samples were not collected for want of clinical suspicion or when BMA is a dry tap. The antibodies used commonly are CD45, MPO, CD20, CD3, CD5, CD10, Tdt, CD34, CD117. CD68 was used when monocyte lineage was suspected. This is in accordance to earlier studies.[6,7] A study on ALL in 2008 showed high concordance between FCM and IHC with CD3 and CD10 in ALL than CD20.[8] Approximate blast count could be done with CD34 and CD117.[9] The 6.97% false negative BMA were reviewed which showed scanty particles with less number of blasts. IHC could estimate the approximate blast percentage as both BMA and BMB were done simultaneously. However CD19 and CD79a were not included in our panel of markers.

BMB was done in almost all suspected cases of myeloma.[10] The antibodies used were CD138, kappa and lambda light chains. IHC had been used to detect plasma cells and monoclonality.[11] In our study IHC was useful in confirming the morphological diagnosis of plasma cell myeloma. Monoclonality for either kappa or lambda light chain was noted (0.9:1 ratio). 21% cases were not suspected clinically. 10.5% BMA gave false negative report of a reactive marrow. IHC done to assess the clonality also helped to diagnose these false negative as well as unsuspected cases. 10.5% cases were clinically suspected to be MM and BMA and BMB also showed high plasma cell count. By IHC these cases were found to be reactive.

Among the lymphomas diagnosed by IHC on BMB 25% cases were received for staging. Only those BMB where

there were diagnostic challenges were subjected to IHC. Identification of bone marrow involvement is highly essential from clinical point of view.[12] A minimum panel of antibodies positive in the lymph node biopsy were used. However, 75% of cases were clinically undiagnosed . As lymphoma was suspected, IHC was done which helped in differentiating lymphoma from non-malignant lymphoid aggregates, diagnostic confirmation and categorization. A study done in 2014 also identified 50% of lymphomas by IHC.[13] To differentiate Hodgkin lymphoma(HL) and non-Hodgkin lymphoma(NHL), the antibodies used were CD45, CD30, CD3, CD20, CD5. The NHL were further categorized by fascin, BCL2, cyclin D1, PAX5, CD10, ALK and EMA. Studies done in the past also preferred to use a panel of antibodies.[14] 55.6% of BMA in these patients were inadequate and 11% cases were false negative.

Metastasis from solid tumors was confirmed in 9% of BMB by IHC. 2 BMB from known prostatic adenocarcinoma (PAC) and another clinically suspected MDS needed IHC confirmation of prostatic carcinoma. The antibodies used for PAC were CD45, vimentin and PSA. The BMB from clinically suspected MDS case was provisionally diagnosed as PAC and confirmed by IHC. In 2013 it was found micrometastasis in PAC in 11.2% of their patients by IHC.[15]The other metastasis were adenocarcinoma from lung, metastatic carcinomas, rhabdomyosarcoma, gastrointestinal tract metastasis and retinoblastoma. In our study primary tumor was identified in 44.4% cases, but in 22.2% cases further metastatic workup beyond IHC was not possible due to economic constraints. Studies have shown BM metastasis from breast cancer, gastric cancer and prostate cancer are common and are prognostically significant.[16] But in our study BMB were not received for many solid tumors. The other 20% BMB included chronic leukemias, MDS, anemic conditions, inflammatory conditions and reactive marrows. IHC was done in MDS for approximate blast count by CD34 and CD117. In two cases of megaloblastic anemia IHC was done with CD45, MPO and glycophorin to differentiate erythroblasts and myeloblasts as the BMA were inadequate with a peripheral pancytopenic picture. IHC was also done to rule out metastasis as isolated tumor cells.[17] IHC for CMV was done for the confirmation of inclusion bodies in one case.

In our study, though IHC was requested in 16.2% it was done on 10.6 % of BMB.IHC helped to confirm AL, MM, lymphomas and solid tumor infiltration in the BM.IHC also helped to diagnose AL, MM and lymphoma which were falsely reported as negative by BMA (6%). IHC was utilized to trace the primary site for malignancy in unknown solid tumors metastasized into BM. IHC was also used to distinguish benign and malignant proliferative conditions and inflammatory diseases.

52(5.6%) biopsies were not subjected to IHC due to economic constraints. In few cases flowcytometry confirmed and categorized AL minimizing the necessity for IHC. The morphological diagnosis in these cases included 73% acute leukemias (n=38), 17% plasma cell myelomas (n=9), 3.8% lymphomas(n=2), 3.8% MDS (n=2), and aplastic anemia(n=1). The MDS and aplastic anemia biopsies required IHC as there were few atypical cells suspicious of blasts. Hence a provisional diagnosis was given in all the above biopsies indicating the necessity for IHC.

Conclusion

IHC is a reliable, robust, technologically standardized procedure which can be performed in BMB. It is a valuable diagnostic tool to confirm and categorize AL especially when the BMA is inadequate for FCM. IHC is a useful technique to differentiate reactive and neoplastic plasmacytosis. IHC is also useful in approximately estimating blast percentage in post chemotherapy AL and MDS. It is better to perform IHC in all BMB with diagnostic challenge for better quality of confirmatory report and patient care.

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