ORIGINAL ARTICLE

Diagnostic Assessment of Bone Marrow Aspiration Smears, Touch Imprints and Trephine Biopsy in Haematological Disorders

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Abstract

Diagnostic Assessment of bone marrow aspirate particle smears, imprints and biopsy sections was done on 40 haematological disorders. Core needle biopsy of the bone marrow is a safe and useful procedure. It is a valuable diagnostic aid for measurement of marrow cellularity and fibrosis.Bilateral trephine biopsy was conducted wherever necessary.Bone marrow aspiration was the most effective method for studying morphological details and was able to diagnose most of the cases except the 2 cases of myelofibrosis which yielded dry tap for which trephine biopsy was diagnostic.Trephine biopsy was also found to be superior for staging of lymphomas and to study the ALIP in MDS.Touch imprints were useful for studying cell morphology, where aspiration yielded dry tap.All the three procedures of bone marrow aspiration, trephine biopsy and touch imprints were found to be complementary to each other and superiority of one method over the other depended on the specific disease process.

Key Words

Hematological, Trephine Biopsy, Bone Marrow Aspiration

Introduction

Marrow biopsy by surgical trephine is an older procedure than needle aspiration. It is only since the late 1950s that core needle biopsy of the bone marrow has been widely used. Since that time, it has had a considerable effect on diagnostic haematology, pathology and oncology. Wide acceptance is associated with the introduction of a simple procedure using the Jamshidi needle to improve the procedure, as well as the quality and size of specimens. The uses and advantages of needle biopsies are numerous. Metastatic deposits, degree of cellularity, fibrosis and assessment of dry taps can readily be determined (1,2).The present study is conducted to evaluate the role of bone marrow aspiration, touch imprints and trephine biopsy in order to optimize diagnostic utility of bone marrow examination which would be of immense value in better patient management

Material and Method

This study has been conducted at the Post Graduate Department of Pathology, Acharya Shri Chander College of Medical Sciences and Hospital, Sidhra, Jammu (J&K). All the patients referred to the Department of Pathology, Acharya Shri Chander College of Medical Sciences and Hospital, Sidhra, Jammu for diagnosis requiring bone marrow examination in haematological disorders were considered for participation in the study. After detailed haematological investigations, the commonly encountered anaemias i.e iron deficiency anaemia, megaloblastic anaemia and haemolytic anaemia were not included.Only those disorders where trephine biopsy is of utility along with bone marrow aspiration were considered eligible for the study.

The standard technique was employed in obtaining the samples from posterior iliac crest using a Jamshidi needle. For preparing the aspirate particle smears, about 0.25 to 0.5 ml of aspirate was obtained into a syringe and delivered onto clean glass slides and smears prepared. The biopsy imprints were made by gently touching the core on slides. The cores were then fixed in Zenkers formalin, decalcified, embedded in paraffin and 2 um thin sections made. The particle smears and biopsy touch preparations were stained by the Wright-Giemsa and the biopsy sections were stained by the Wright-Giemsa and haematoxylin

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and eosin methods. In addition Gomori's reticulin stain and prussian blue stain for iron were also performed

Results

The different haematological disorders encountered are enumerated in *table No-1*

Table 1. Haematological Disorders

Disorders	No. of patients	%age
Acute leukaemia	20	50
Chronic Myelocytic leukaemia	4	10
Chronic Lymphocytic leukaemia	2	5
Idiopathic thrombocytopenic purpura	2	5
Multiple myeloma	4	10
Myelofibrosis	2	5
Aplastic anemia	2	5
Myelodysplastic syndrome	1	2.5
Lymphomas	3	7.5
Total	40	100

Amongst the primary haematological disorders-acute leukaemias formed the largest group accounting for 20(50%) cases of which 12 cases were categorized as AML & 8 as ALL. The next in the descending order of frequency were CML & multiple myeloma 4(10%) cases each, lymphomas 3(7.5%) cases followed by CLL, ITP, myelofibrosis, aplastic anemia 2(5%) cases each and myelodysplastic syndrome 1(2.5%) case. Out of the 3 cases of lymphomas, 2 cases were of Non-Hodgkin lymphoma and 1 case was of Hodgkin lymphoma

Haematological parameters provided valuable information about various disorders. e.g the finding of marked rouleux formation along with the presence of plasma cells and markedly increased ESR pointed to the provisional diagnosis of multiple myeloma. Tear drop cells along with leukoerythroblastic blood picture pointed towards Myelofibrosis if concomitant splenomegaly was present.The finding of increased myelocytes, metamyelocytes, band and segmented forms of neutrophils along with increased basophilia and increased platelet count pointed towards CML.

Peripheral blood lymphocytosis along with smudge cells gave the impression of CLL. Acute leukaemias were easily detected by the presence of myeloblasts or lymphoblasts in the PBF.Pancytopenia with reticulocytopenia indicated aplastic anaemia while severe thrombocytopenia with presence of activated giant platelets in the backdrop of viral infection was strongly suggestive of ITP.Pancytopenia with dyserythropoiesis & dysmyelopoiesis was a feature of MDS. Two of the three cases of lymphoma were diagnosed on lymph node HPE as NHL-Intermediate grade, while one was a case of Hodgkins disease mixed cellularity type. Bone marrow examination in all the three cases was performed for the purpose of staging. In one case of NHL, a few atypical monocytoid lymphoid cells were observed on PBF. In the other two cases of lymphoma(one NHL & one Hodgkin disease),PBF revealed a normocytic normochromic anaemia with mild reduction in platelet count

Once the baseline haematological parameters were recorded, all the 40 patients were subjected to bone marrow examination. Twin procedures of bone marrow aspiration & trephine biopsy were performed in each case and touch imprints were prepared from each trephine biopsy core specimen prior to processing.It was observed that imprint smears provided a diagnosis much earlier than the trephine biopsy specimen, which takes longer time to process. Marrow histological sections were examined for architecture, cellularity, presence of foreign cells, granulomas, marrowfibrosis, dyserythropoiesis and dysmegakaryopoiesis.

2 patients (5%) in whom bone marrow aspiration yielded dry tap, were diagnosed as myelofibrosis on trephine biopsy. Trephine biopsy was also superior to bone marrow aspiration in the 3 cases(7.5%) of lymphomas, 2 cases(5%) of aplastic anaemia and 1 case(2.5%) of CLL. Trephine biopsy also proved better than bone marrow aspiration in the diagnosis of Myelodysplastic syndrome (2.5%) since it showed the presence of aggregates of immature myeloid precursor cells. Bilateral trephine biopsy was necessary in diagnosing 2 cases (5%) of NHL which could not be detected on unilateral trephine biopsy. Both bone marrow aspiration and trephine biopsy were complementary in 20 cases(50%) of acute leukaemias,4 cases(10%) of CML,2 cases(5%) of CLL,4 cases(10%) of multiple myeloma,1 case(1.5%) of myelodysplatic syndrome, 3 cases(7.5%) of lymphomas, 2 cases(5%) of ITP and 2 cases(5%) of aplastic anaemia. All the 3 procedures were found to be complementary in all the cases of leukaemias, multiple myeloma, lymphomas and MDS.The iron content in bone marrow aspiration smears and bone marrow trephine biopsy sections using Perls' stain is shown in *table no. 2*

Our study showed that that aspirate films were more sensitive than trephine biopsy sections for the detection of haemosiderin when the biopsy specimens were decalcified in formic acid.

They also provided a more accurate reflection of bone marrow iron stores, because decalcification led to an unquantifiable loss of iron. Also in 2 cases of myelofibrosis where aspiration yielded a dry tap, trephine biopsy was essential for detection of iron

Table 2. The Iron Content in Bone Marrow Aspiration Smears and Bone Marrow Trephine Biopsy Sections Using Perls' Stain

Disorder	Iron stores
AML(n=12)	Normal iron stores(2+ to 3+) (n=10) Reduced iron stores(0+ to 1+) (n=2)
ALL(n=8)	Normal iron stores(2+ to 3+) (n=7) Reduced iron stores(0+ to 1+) (n=1)
CML(n=4)	Normal iron stores(2+ to 3+) (n =4)
CLL(n=2)	Normal iron stores(2+ to 3+) (n =2)
ITP(n=2)	Normal iron stores(2+ to 3+) (n =2)
Multiple myeloma(n=4)	Normal iron stores(2+ to 3+) (n =4)
Myelofibrosis(n=2)	Reduced(0 to 1+) (n=2)
Aplastic anaemia(n=2)	Reduced iron stores(0 to 1+) (n=2)
Myelodysplastic syndrome(n=1)	Increased iron stores 5+ (n=1)
Lymphomas(n=3)	Normal iron stores(2+ to 3+) (n =3)

Discussion

In our study of the Haematological disorders , acute leukaemia was observed to be the most common disorder.In the present study, both bone marrow aspiration and trephine biopsy were complementary in all the 20 cases of acute leukaemias, 4 cases of CML, 2 cases of CLL, 4 cases of multiple myeloma, 2 cases of ITP, 2 cases of aplastic anaemia, 1 case of myelodysplatic syndrome and 3 cases of lymphomas.While aspiration smears were observed to be most effective for studying cellular morphology, biopsy on the other hand, was helpful in assessing marrow cellularity and for diagnosing cases of aplastic anemia, myelofibrosis, Hodgkin's disease. Our findings are comparable to the study by James *et al* (1) who observed that combined procedures of aspiration and biopsy gave a higher yield and are essential in patients with non-Hodgkin's lymphoma and Hodgkin's disease. In a study it was observed that bone marrow aspiration is frequently not helpful in the diagnosis of aplastic anaemia, myelofibrosis, Hodgkin's and non-Hodgkin's lymphoma , but it is important that both aspirated and biopsy material should be examined together, since the two methods are often complementary (2).

In our study, bone marrow aspiration, touch imprints and trephine biopsy were found to be complementary in all the cases of leukaemias, multiple myeloma, lymphomas and MDS. We observed that fibrosis of bone marrow, pattern of bone marrow involvement and topographical alterations were appreciable only on trephine sections as has also been noted by Varma *et al* (3) who opined that although trephine sections provide maximum information, all three preparations complement each other and should be evaluated simultaneously for complete bone marrow interpretation.Our study of haematological disorders showed that 2 cases (5%) in whom bone marrow aspiration yielded dry tap, were diagnosed as myelofibrosis on trephine biopsy. Trephine biopsy was also superior to bone marrow aspiration in diagnosing 3 cases (7.5%) of lymphomas and 2 cases (5%) of aplastic anaemia. Trephine biopsy was also better than bone marrow aspiration in the diagnosis of Myelodysplastic syndrome since it showed the presence of aggregates of immature myeloid precursor cells.

Bettini *et al* (4) observed that bone marrow biopsy in chronic myeloproliferative disorders provides independent diagnostic and prognostic data. In a study Sabharwal *et al* (5) included 7 cases (23.3%) of myelofibrosis which were diagnosed on trephine biopsy sections. Humphries (6) in his study of 87 cases of dry tap on marrow aspiration, obtained trephine biopsies which showed significant pathology. Dry tap with marrow aspiration were due to fibrosis and hypercellularity. The frequent diseases diagnosed on bone marrow biopsy were metastasic cancers, chronic myeloid leukaemia, idiopathic myelofibrosis and hairy cell leukaemia. We also observed 2 cases (4%) of myelofibrosis which yielded dry tap on bone marrow aspiration which were subsequently diagnosed on trephine biopsy. Hence the finding of a dry tap should never be dismissed as being due to faulty technique and always needs a bone marrow biopsy. In a study, Ozkalemkas *et al* (7) observed that bone marrow biopsy is certainly indicated whenever aspiration results in an insufficient material especially in the presence of microangiopathic hemolytic anemia (MAHA), leukoerythroblastosis (LEB) and unexplained cytopenias. In our study too, Leukoerythroblastosis in PBF was a definitive indication for bone marrow biopsy especially when aspiration yielded dry tap.The present study included four cases of multiple myeloma. In all the 4 cases good cellularity was obtained on aspiration and an accurate diagnosis was easily arrived at.

However, Sabharwal *et al* (5) in their study observed that aspiration smears provided inadequate information for accurate diagnosis of multiple myeloma and it was only the biopsy imprint smears which showed marrow infiltration by sheets of mature and immature plasma cells. Orazi A (8) observed the role of bone marrow biopsy in MDS, which showed the presence of aggregates or clusters of immature myeloid precursor cells (myeloblasts and promyelocytes). He concluded that myelodysplastic/ myeloproliferative disorders can only be accurately

categorized by a careful multiparametric approach in which the bone marrow biopsy exerts a pivotal role. This is in concordance with our study where abnormal localization of immature precursors could be observed only on trephine biopsy sections though features of abnormal erythropoiesis and myelopoiesis were easily detected on aspirate smears as has also been obseved by Sabharwal *et al* (5).

Three cases of lymphomas - two NHL and one Hodgkin disease - were included in the present study and all the three cases reflected a 100% positive result for both aspirate smears and biopsy sections. However, trephine biopsy by virtue of providing architectural pattern was superior for the staging and exact categorization of lymphomas. Sabharwal *et al* (5), in their study, also recorded a 100% positive result for aspiration smears and biopsy sections in the two cases of lymphomas studied by them. Kar *et al* (9) observed that the bone marrow may sometimes be the only or initial site of involvement in Hodgkin's lymphoma. Therefore, it is important to pick up the histopathological findings of Hodgkin's lymphoma in a bone marrow trephine section with a normal aspirate and with absence of any clinical indicators.Mostserrat *et al* (10) observed that although bone marrow histological pattern and bone marrow aspiration are of value to estimate bone marrow lymphocytic infiltration in chronic lymphocytic leukaemia but to predict the outcome of the disease, bone marrow biopsy is more reliable than aspiration. We also noted in our study that in one of the two cases of CLL, trephine biopsy was superior in assessing the pattern and degree of lymphocytic infiltration in the bone marrow

In the present study it was obseved that amongst the haematological disorders bilateral trephine biopsy was diagnostic in 2 cases of NHL which were missed on unilateral trephine biopsy. Menon and Buchanan (11) observed that bilateral trephine biopsy was superior to unilateral biopsy for the demonstration of bone marrow involvement by Hodgkin's disease or non-Hodgkin's lymphoma and recommended that bilateral trephine biopsies should be performed when knowledge of the state of the bone marrow was important for clinical decision making.

The present study also attempted to comparatively evaluate the quality of Perl's staining for iron on aspiration films, imprint smears and biopsy sections. It was observed that aspirate films were more sensitive than trephine biopsy sections for the detection of haemosiderin and also provided a more accurate reflection of bone marrow iron stores as the biopsy specimens were decalcified in formic acid. However, in the 2 cases of myelofibrosis, where aspiration yielded a dry tap, trephine biopsy became essential for detection of iron. Stuart-Smith *et al* (12) also observed that the aspirate films were more sensitive than trephine biopsy sections for the detection of haemosiderin and assessment of bone marrow iron stores, because decalcification led to an unquantifiable loss of iron.

Conclusion

All the three procedures of bone marrow aspiration, trephine biopsy and touch imprints were found to be complementary to each other and superiority of one method over the other depended on the specific disease process.

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