

Bone marrow examination - A clinicopathological study of 150 patients in a tertiary care hospital at Guntur (AP)

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Abstract

Background: Bone marrow examination is generally indicated for staging, diagnosis, therapeutic and prognostic monitoring of various hematological and non hematological disorders. In recent times there was study rise in bone marrow indication for follow up cases than for initial diagnosis. It is important not only for morphological evaluation but also for ancillary studies which provides valuable data for diagnostic and therapeutic endeavors. The main aim of our study is to find out changing trends in indications of bone marrow study in a tertiary care center. **Methods:** Fifty seven cases for which we have done both aspiration and biopsy were studied and findings are compared morphologically. Later in some cases where ever was possible the morphological diagnosis is correlated with ancillary studies and recorded the findings. Pathological diagnosis was correlated with clinical indications of bone marrow examination. **Results:** Among age groups most of the patients were between 41-50 years and male to female sex ratio was 1.11:1. Most of the bone marrows were performed for pancytopenia work up accounting for 22.80%. Over all 28% were indicated for staging 25% were done for initial diagnosis and 47% were done for follow up/therapeutic response. On microscopic examination most common disease diagnosed in our study was 26.31% cases of iron deficiency anemia and among malignant hematological disorders majority was NHL with 7%. **Conclusion:** There is an increased incidence of bone marrow examination not only for morphological study but also for ancillary tests like flow cytometry, immunohistochemistry and molecular studies which can give more precise and molecular based diagnosis for clinician to reach their therapeutic and prognostic goals. In our study we noted core biopsy is more accurate but aspiration and biopsy both procedures have their own merits and demerits in the aspect of rapidity, accuracy and utilization of material for ancillary studies.

Keywords: Ancillary studies, Bone marrow morphology, changing trends, Indication.

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Introduction

Bone marrow examination is very useful not only in the diagnosis of hematological disorders but also useful in diagnosis of systemic diseases like storage diseases, pyrexia of unknown origin, granulomatous diseases and metastatic diseases[1]. The diagnoses of hematological disorders were mainly done by taking complete clinical history, careful physical examination, complete haemogram and bone marrow examination [2,3].

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Bone marrow examination can yield two types of specimens bone marrow biopsy and aspirate. The biopsy specimen is best

for the architectural evaluation and its relation with pathologic marrow elements[3,4]. Core biopsy is best for ancillary study like immuno-histochemistry. The aspirate specimen is good for cytomorphology, for differential cell counts and for flow cytometry, cytogenetics, and molecular tests[5,6] The rapidly evolving knowledge about haematological malignancies and changing therapeutic trends basing on ancillary studies the bone marrow examination has become more critical for all practical purposes. Improvements in bone marrow aspirate and needle technology has made the procurement of adequate specimens more reliable and efficient[5-7].

Methods

Ethical clearance was obtained from institutional ethics committee to study patients and examine the material

available at Government General Hospital, Guntur and department of pathology, Guntur Medical College, Guntur from January 2019 to December 2019 for a period of one year. During our study period a total of 150 patients were referred for bone marrow examination. Among those patients we have included only the patients who have undergone both bone marrow aspiration and biopsy in our study. After extraction of bone marrow material we have excluded acellular and hypocellular marrows obtained due to poor technical reasons. Finally we have studied fifty seven cases for which we have both aspiration and biopsy with cellular material. We have applied percentage data statistical analysis to study this group of patients. For every patient relevant clinical history, clinical examination details, radiological findings were noted. Patients were investigated for complete blood count, coagulation profile, reticulocyte count and peripheral smear examination. Then patients were subjected to bone marrow examination after informed consent. The decision for bone marrow aspiration or biopsy made basing on clinician opinion, after the assessment of the information available and condition of patient. To evaluate mono and bicytopenias (anemias, thrombocytopenia), aspiration is helpful and biopsy is not mandatory. In the cases of pancytopenia both aspiration and biopsy are mandatory. In malignant hematological diseases like MDS, acute leukemias, Lymphomas except SLL/CLL B type, Myeloproliferative diseases, Myelofibrosis, plasma cell disorder both aspiration and biopsy were done. In acute leukemias aspiration can be sufficient for morphological diagnosis but biopsy will be done in case of dry tap or reduced cell count in the aspirate. But in CLL bone marrow examination is not indicated and immunophenotyping on peripheral blood can be diagnostic. In the case of CML biopsy is done when if the BCR-ABL fusion gene or the Philadelphia chromosome cannot be detected in the peripheral blood or if enough material cannot be aspirated. In the case of dry tap in aspiration, metastatic diseases, granulomatous disease, Hodgekins disease, systemic diseases like storage diseases, bone disorders bone marrow biopsy is indicated than aspiration. In diseases like leishmaniasis aspiration is mandatory and biopsy is usually not indicated. We preferred posterior superior iliac crest for obtaining bone marrow in adults and children because it contains cellular marrow, no vital organs are in close proximity and it is a nonweight-bearing structure. After infiltrating 1% xylocaine as local anesthetic and saline's bone

marrow needle was used to do aspiration. Where ever indicated aspirated material was transferred in to heparinised vacutainers for ancillary studies like flow cytometry and molecular analysis. The remaining material smeared and stained with leishmans stain along with peripheral smear. Bone marrow biopsy was taken by Jamshidi needle and specimens were fixed in 10% formalin. After decalcification in 10% formic acid and 5% formaldehyde, material was subjected to routine histopathology processing and stained by Hemotoxylin and Eosin stain. Special stains and immuno-histochemical markers were applied wherever indicated. We selected only cases with adequate material for study and excluded cases which were commented inadequate or not suitable for assessment due to hypocellularity, clotted blood, diluted, showing artifacts due to anticoagulant e.t.c. Finally we included fifty seven cases for which we have done both aspiration and biopsy. Both bone marrow biopsy and aspiration findings were studied and findings are compared morphologically. For the benefit of patient and if there is a clear indication aspirated material was sent to higher center for ancillary studies. Later in some cases where the tracking is possible the morphological diagnosis is correlated with ancillary studies and recorded the findings.

Results

A total number of 57 cases were studied during the study period from January 2019 to December 2019. Among age groups most of the patients were between 41-50 years accounting for 22.80% (13 cases). 61-70 years were the next commonly effected group with 19.29% (11 cases). Patients age range from 4 years old child to 78 years old patient. Thirty cases were male patients and twenty seven were female with sex ratio 1.11:1 which is almost equal (Table 1). Clinical indications for bone marrow are mainly for staging of disease, for initial diagnosis and for follow up to know therapeutic response and prognostic significance. Most of the bone marrows were performed for pancytopenia work up accounting for 22.80% (13 cases). Next common indication for bone marrow was staging of 9 lymphoma cases accounting for 15.78%. Anemia and thrombocytopenia accounts for 14.03% (8 cases) each. Indication for diagnosis of leukemia accounts for 5.26% (3) only. Over all 28% were indicated for staging 25% were done for initial diagnosis and 47% were done for follow up/therapeutic response (Table 2).

Table 1: Distribution of cases according to age and sex

Age	Male	Female	Total
1-10	3	1	4 (7%)
11-20	0	7	7 (12.28%)
21-30	2	2	4(7%)
31-40	6	3	9(15.78%)
41-50	8	5	13(22.80%)
51-60	1	3	4(7%)

61-70	7	4	11(19.29%)
71-80	3	2	5(8.77%)
Total	30	27	57

Table 2: Indications for bone marrow study

S.No	Indications	No .of Cases	%
1	Bicytopenias and pancytopenias	13	22.80%
2	Lymphoma for diagnosis and staging	9	15.78%
3	Anemia for evaluation	8	14.03%
4	Thrombocytopenia	8	14.03%
5	Fever for evaluation	5	8.77%
6	Leukemia	3	5.26%
7	Plasma cell disorder	3	5.26%
8	Back pain	2	3.50%
9	Weakness	2	3.50%
10	MDS	1	1.75%
11	Secondaries	1	1.75%
12	Lymphadenopathy	1	1.75%
13	Myeloproliferative/Polycythemia	1	1.75%
	Total	57	100%

On microscopic examination 44(77.20%) cases were grouped under non-malignant hematological disorder, 12(21.05%) cases were malignant hematological and one (1.75%) was non hematological malignant case. Among all cases majority were non malignant hematological disorders and most common disease diagnosed in our study was 15(26.31%) cases of iron deficiency anemia and 2nd most common was 10(17.5%) cases of megaloblastic anemia. 6(10.5%) cases of

idiopathic thrombocytopenic purpura was diagnosed. Among malignant hematological disorders majority was NHL with four cases(7%), next common was Plasma cytomawith three cases(5.25%). To know the diagnostic merits and demerits of bone marrow aspiration and biopsy we have compared and analyzed the diagnoses made on both procedures for all cases(Table 3).

Table 3: Pathological diagnosis of cases -correlation between aspiration and biopsy

1. Malignant Hematological Disorders			
	No. of Cases	Diagnosis in Aspiration	Diagnosis in Biopsy
ALL	1(1.75%)	ALL	ALL
Acute Leukemia	1(1.75%)	Dry tap	Acute leukemia
SLL/CLL	1(1.75%)	Dry tap	CLL
CML	1(1.75%)	CML	CML
Plasma cell Disorder	3(5.25%)	Plasma Cell Disorder(3 cases)	Plasma Cell Disorder(3 cases)
Hodgekins Lymphoma	1(1.75%)	Hodgekins Lymphoma	Hodgekins Lymphoma
NHL	4(7%)	NHL(3 cases)	NHL(4 cases)
		Scanty Material(1 case)	
2. Non-Malignant hematological disorders			
Megaloblastic Anemia	10(17.5%)	Megaloblastic Anemia(9 cases)	Megaloblastic anemia(10 cases)

			Normal Study(1 case)	
	Aplastic Anemia	1(1.75%)	Very Scant Cellularity	Aplastic anemia
	ITP	6(10.5%)	ITP(6 cases)	ITP(6 cases)
	Iron deficiency Anemia	15(26.31%)	IDA (13 cases)	IDA(15 cases)
			Normal Study(2 cases)	
	Reactive marrow/Erythroid Hyperplasia	8(14%)	Reactive marrow/Erythroid Hyperplasia(8 cases)	Reactive Marrow/Erythroid Hyperplasia(8 cases)
	Normal Study	4(7%)	Normal Study(4 cases)	Normal Study(4 cases)
3	Non hematological diseases effecting bone marrow			
	Secondaries	1(1.75%)	Secondaries (1 case)	Secondaries(1 case)

Among non malignant hematologic disorders among 10 cases of megaloblastic anemia 9 were diagnosed with aspiration and one case was diagnosed as normal study. But in biopsy all 10 cases were confirmed as megaloblastic anemia. 15 cases of iron deficiency cases 13 were diagnosed in aspiration 2 were diagnosed as normal study. But all 15 cases were reported as iron deficiency anemia in trephine biopsy. Aplastic anemia shows scanty cells on aspiration diagnosed in trephine biopsy. ITP, reactive hyperplasia were diagnosed in both aspiration and biopsy. In both aspiration and biopsy 4 cases were diagnosed as normal study. Among malignant hematological diseases nine cases were diagnosed both by aspiration and trephine biopsy. One case of acute leukemia, one case of CLL did not yield material on aspiration, one case of NHL showed scanty cellularity in aspiration and in all cases diagnosis were made with the help of trephine biopsy. One non hematological malignancy was

diagnosed with aspiration and biopsy with support of ancillary study. On clinicians request in the view of therapeutic and prognostic reasons bone marrow material of seven malignant hematological cases were subjected to ancillary studies like flow cytometry, immune-histochemistry and molecular genetics within our center and at higher center basing on availability of tests. We have followed these cases and noted the results and compared with our morphological diagnosis. One case of NHL which was diagnosed morphologically as NHL was diagnosed as B-ALL in the flow cytometry. Another case of acute leukemia diagnosed more specifically as AMLM1 in flow cytometry, the remaining 4 cases were diagnosed in flow cytometry as same as the morphology (Table 4). The bone marrow material was sent for ancillary studies to detect BCR-ABL fusion gene for a CML case.

Table 4: Malignant Hematologic Diseases - Correlation of Morphological Diagnosis with Ancillary Tests

S.No	No. of Cases	Bone Marrow Morphological Diagnosis	Ancillary Tests
1	1	ALL	B-ALL in Flow Cytometry
2	1	SLL/CLL	CLL in Flow Cytometry
3	1	Acute Leukemia	AML-M1 in Flow Cytometry
4	1	Plasma cell Disorder	Plasma cell disorder in Flow Cytometry
5	1	NHL	B-ALL in Flow Cytometry
6	1	Lymphoma infiltrating Marrow	LBCL in Flow Cytometry
7	1	CML	Molecular Genetics

In our study we noted core biopsy is more accurate than aspiration and it is useful in some cases where aspiration

failed to yield material. But both procedures have their own merits in the aspect of rapidity, accuracy and utilization of

material for ancillary studies. Ancillary studies can give more precise and molecular based diagnosis for clinician to reach their therapeutic goals.

Discussion

Bone marrow aspiration and biopsy is a rapid and essential procedure to know the required information regarding the marrow of the patient. It is safe and easy procedure that can be performed on any age of patient. In our study most of the patients were between 41-50 years accounting for 22.80%. Patients age range from 4 years old child to 78 years old patient. But Merzianu M et al. in their study mentioned average was 52 years and age range was 1-102 years[8]. Qahtani FSA et al. noted patients age range from 5 weeks to 99 years, with a mean age of 38 years in their study[9]. Thirty cases were male patients and twenty seven were female with sex ratio 1.11:1 which is almost near to 1.27 as noted by Merzianu M et al. and 1.4:1 mentioned in Qahtani FSA et al. study[8,9]. The indication for bone marrow examination is very wide. Clinicians indicate bone marrow predominantly for diagnosis of diseases, staging of diseases and as a follow up tool[10,11]. In our study we noted most of the bone marrows were performed for pancytopenia work up accounting for 22.80%. Indication for diagnosis of leukemia accounts for 5.26% only. Over all 28% were indicated for staging 25% were done for initial diagnosis and 47% were done for follow up/therapeutic response and for ancillary studies. Merzianu M et al. in their study noted that 23% were indicated for staging and 77% were for nonstaging and among non staging indications 44% performed for initial diagnosis and 52% for follow-up[8,10]. But Qahtani FSA et al. in their study noted that the frequent indications or referrals of bone marrow examination were 18.5% for diagnosis or management of acute leukemia, 15.5% for staging of lymphoma, 13.09% for work up of pancytopenia and remaining were for other reasons[9,10]. We noted that there is changing indication for bone marrow examination which is more commonly indicated for staging, ancillary studies and follow up than diagnostic in tertiary care centers. This could be due to patients are coming to tertiary care centers after the diagnosis and increased availability of ancillary tests like flow cytometry, molecular studies and immunohisto-chemistry at higher centers[5-7,11]. On morphological examination in our study 44(77.20%) cases were non malignant hematological disorder, 12(21.05%) cases were malignant hematological and one(1.75%) was non hematological malignant case. Our findings are almost similar to study done by Gandapur ASK et al. who observed 73.2% cases were non-malignant haematological conditions while 27.8% cases were different haematological malignancies[12]. But Qahtani FSA et al. in their study reported 82.5% cases with malignant hematological disorders and 13.5% were non-malignant hematological conditions on bone marrow morphological examination. Remaining 4% were non-hematological diseases[9]. Merzianu M et al. in their pathological diagnosis 44% were negative for neoplasm, 5% non-neoplastic, 42% malignant hemato-logic diseases, remaining were inconclusive and inadequate specimens[8]. Among all cases and in non malignant category most common disease diagnosed in our study was 15(26.31%) cases of iron

deficiency anemia and 2nd most common was 10(17.5%) cases of megaloblastic anemia. Next was idiopathic thrombocytopenic purpura with 6(10.5%) cases. Similar findings were reported by Kaur M et al., Sima Chauhan et al., AtlaBL et al. and Gandapur ASK et al [1,2,4,12] But Merzianu M et al noted leukemia/myeloid neoplasms were the most common group in pathological diagnosis with 23% of total bone marrow examination. Next common group was plasma cell neoplasms with 10%, followed by lymphoma with 6% [8,12]. Qahtani FSA et al. also in their study noted acute leukemia with 15% was most common marrow finding on microscopy hematological malignancy, followed by 10.3% cases of myeloproliferative disorders and 4.7% of multiple myeloma. In non-malignant hematological conditions 3.5% were idiopathic thrombocytopenic purpura followed by anemia 3.1% [9]. In our study among malignant hematological disorders 4(7%) of NHL was reported in four cases, 3(5.25%) cases of Plasma cytoma in three cases both in aspiration and biopsy. We reported 2(3.50%) cases of acute leukemia. Possible reasons for higher incidence of iron deficiency and megaloblastic anemia in our setup is dietary factor (lack of iron and Vitamin B12) due to poverty as majority patients coming to government hospital belong to poor socioeconomic strata.

Among 12 malignant hematologic disorders 7 cases were sent for ancillary studies like immunohisto-chemistry, flow cytometry and molecular genetics on clinicians request. Except one case which was diagnosed morphologically as NHL was diagnosed as B-ALL in flow cytometry. Another case of acute leukemia diagnosed more specifically as AMLM1 in flow cytometry, the remaining 4 cases were diagnosed in flow cytometry as same as the morphology. One case of CML was sent for ancillary studies to detect BCR-ABL fusion gene in molecular studies. This supports the importance of morphological diagnosis were the ancillary studies are not available. So bone marrow aspiration and biopsy still plays important role in malignant hematological diseases and should used in a complement manner to arrive at confirmative diagnosis. Ancillary studies can give more precise and therapy oriented results use full to clinician to treat and follow up of patient [13-16].

Conclusion

Generally clinicians indicate bone marrow examination depending upon changes in the peripheral blood, in suspicion of a diagnosis or to know therapeutic prognosis/staging of disease in a previously known case. With changing trends clinicians also indicate bone marrow examination for ancillary tests based on flow cytometry, immunohistochemistry and molecular studies. In our centre major clinical indications of bone marrow examination were for evaluation of pancytopenia, anemia and for staging of malignant disease. Pathological diagnosis we commonly encountered was nutritional anemia, ITP, and NHL. Bone marrow evaluation for multiple myeloma also increased. In our observation we noted that biopsy is more superior to aspiration for morphological diagnosis and both procedures have their own merits and demerits in the aspect of rapidity, accuracy and utilization of material for ancillary

studies. We also noted increased importance of ancillary studies which gives more precise information in the view of therapeutic and diagnostic endeavors which are useful in treating patient.

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