Section: Pathology

Research

STUDY OF HISTOMORPHOLOGICAL PATTERNS IN BONE MARROW ASPIRATION FAILURE CASES

Sharique Ahmad[1], Surekha Kadadi[2], Swati Handralmath[3], Ahmad Raza[4], Nishtha Bassi[4], Iqra Sheikh[4]

[1]Professor, Department of Pathology, Era's Lucknow Medical College and Hospital, Era University, Lucknow, Uttar Pradesh, India.
[2]Associate Professor, Department of Pathology, Ashwini Rural Medical College and Hospital & Research Centre, Kumbhari, Solapur, Maharashtra, India.
[3]Assistant Professor, Department of Pathology, Ashwini Rural Medical College and Hospital & Research Centre, Kumbhari, Solapur, Maharashtra, India.
[4]Junior Resident Department of Pathology, Era's Lucknow Medical College and Hospital, Era University, Lucknow, Uttar Pradesh, India.

Received : 20/12/2022
Received in revised form : 17/01/2023
Accepted : 31/01/2023

Keywords:
Bone marrow aspirations, Trephine Biopsy, histomorphological, Leukaemia.

Corresponding Author:
Dr. Sharique Ahmad
Email: diagnopath@gmail.com
ORCID: 0000-0003-4701-6109

DOI: 10.47009/jamp.2022.5.1.136

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2023; 5 (1); 648-652

Abstract

Background: Bone marrow examination is a useful investigation for diagnosing a large number of haematological and non-haematological disorders. Bone marrow aspiration provides relevant information about the numerical and cytological features of marrow cells whereas bone marrow biopsies provide excellent appreciation of spatial relationship between cells and of overall bone marrow structure. Bone marrow cytology and trephine biopsy histopathology complement each other and the superiority of one method over the other depends on the underlying disorder.

The present study was carried out in the Department of Pathology, Era's Lucknow Medical College and Hospital, Era University, Lucknow to see the histomorphological patterns of trephine biopsy in bone marrow aspiration failure cases. Total 50 patients of aspiration failure cases were trephined to obtain bone marrow from posterior iliac crest during the period of two years from April 2017 to March 2019, attending the departments of haematology and medicine, ELMC&H, Lucknow. In four cases (8%), trephine biopsy failed to show any marrow tissue and were discarded from the present study. The rest 46 cases (92%) with adequate biopsy material revealed 19 cases (41.30%) of acute leukaemia, 13 cases (28.26%) of chronic myeloproliferative disorders, 07 cases (15.22%) of hypoplastic marrow, 02 cases (4.34%) of non-Hodgkin's lymphoma and one case (2.17%) of each of multiple myeloma, reactive marrow and metastatic carcinoma. Out of 46 cases, diagnosis could not be done in two cases (4.34%) due to poor preparation of biopsy material. In similar type of studies, Navone and Colombano[6] reported 16%(35 out of 228) acute leukaemia, 9.8%(44 out of 445) chronic myeloproliferative disorders, 8.8%(29 out of 328) malignant lymphoma and Hodgkin's diseases, 4.8 %(10 out of 208) myelomas and 13.5%(10 out of 74) metastatic carcinomas in trephine biopsy of bone marrow aspiration failure cases. The discordance between these studies may be due to difference in their sample size. In the present study, two cases remained undiagnosed and five cases of acute leukaemia could not be subcategorized into AML or ALL. It appears that as only H&E and reticulin stains were used in this study, those cases could not be diagnosed or subcategorized due to lack of other facilities. So it is recommended that facilities for plastic embedding, immunocytochemistry and use of enzyme and immunophenotyping should be developed in the department and used whenever necessary.

INTRODUCTION

Bone marrow examination plays a pivotal role in diagnosing a variety of haematological and non-haematological disease. It is well known that the blood picture does not always reflect abnormalities accurately that may exist in blood forming organs.[1] The histopathological study of Bone marrow trephine biopsy gives well-preserved marrow architecture of the bone marrow with its all cellular and stromal components. Therefore, for the diagnosis of aplastic anemia, metabolic bone disease, Myelofibrosis and

ISSN (O): 2687-5365; ISSN (P): 2753-6556

granulomatous involvement trephine biopsy becomes necessary,.[2] Sing et al.[3] gave their opinion that marrow examined can be utilized and considered to detect metastatic tumour when other techniques are negative for making a tissue diagnosis of suspicious lesions, especially if a primary site is unknown. They also mentioned that the diagnosis in a majority of cases is established by Bone marrow biopsy than aspirate. Brynes et al.[4] have stated the importance of examination of bony trabeculae for diagnosing metabolic disorders such as osteoporosis, Paget's disease, primary and secondary hyperparathyroidism. The lymphoma and benign lymphoid aggregates can be differentiated by distinguishing marrow invasion through the relationship of haemopoietic cells to the bony trabeculae. According to Lee et al.[5] trephine biopsy of bone marrow is the answer for the diagnosis of the diseases where there is repeated failure of marrow aspiration. It is to be noted that no study on trephine biopsy of bone marrow has been done yet in our country. This study was done to see the histomorphological patterns of trephine biopsy in aspiration failure cases.

MATERIALS AND METHODS

This study was performed in the Department of Pathology at Era's Lucknow, medical College and Hospital, Era university, Lucknow during the period from April, 2017 to March, 2019. A selection of 50 patients of different sex and age were selected from the departments of haematology and medicine of ELMC&H, Lucknow. Clinically diagnosed patients of haematological and non-haematological disorders supported by relevant laboratory investigations were subjected to bone marrow aspiration and the cases in whom bone marrow aspiration failed repeatedly were selected for trephine biopsy. The bone marrow biopsy was taken from either of the posterior iliac spines. Islam's trephine biopsy needle was used for this purpose. In the department of pathology, gross examination of the specimen was done. It was then placed in a bottle containing 10% buffered formalin and kept overnight for proper fixation. In the next morning, the specimen was washed in water for 30 to 60 minutes. Short decalcification of the specimen was done with 10% nitric acid for about one hour. Then it was washed for 60 to 90 minutes with several changes of water and submitted for tissue processing with paraffin impregnation. Routine paraffin section were stained with haematoxylin and eosin staining method and examined under light microscope. When necessary tissue sections were also stained with some special stains (e.g. reticulin) for accurate diagnosis. Specimens containing at least five marrow spaces in any section were considered adequate for histopathological diagnosis.

RESULT

Out of 50 cases, trephine biopsy failure occurred in four cases. Out of these four cases, in two cases, bone marrow could not be trephine out, rather only cortical bone without marrow were obtained, and in one only marrow material without bone or bony trabeculae and yet in another, marrow spaces contained only adipose tissue but no haemopoietic element. Clinically out of these four cases, two were acute leukemia, one chronic leukemia and the other one was aplastic anaemia. Clinical diagnoses of the rest 46 cases are shown in [Table 1].

Table 1: Clinical diagnoses of 46 cases bone aspiration failed

Clinical diagnosis	Total
Acute leukemia	19
Chronic myeloid leukemia	10
Chronic lymphocytic leukemia	01
Myelofibrosis	03
Hypoplastic anemia	05
Kala-azar	03
Multiple myeloma	02
Lymphoma	01
Combined deficiency anemia	02
Total	46

Each of the specimens of 46 trephine biopsies of bone marrow was 2 mm in diameter. Their length ranged from 0.3, to 1.6 cm. Of the 46 biopsies, on histological basis, acute leukemia was found in 19 cases, which comprised 41.30% of the total. Chronic myeloproliferative disorders were found in 13 cases (28.26%), hypoplastic anaemia in 07 cases (15.22%), 02 cases (4.34%) of Non-Hodgkin's Lymphoma, 02 cases (4.34%) of undiagnosed lesion and one patient each (2.17%) of multiple myeloma, reactive marrow and metastatic carcinoma (poorly differentiated). Histological diagnosis of trephine biopsy of bone marrow of 46 cases of aspiration failure is shown in [Table 2].

Table 2: Histopathological diagnosis of trephine biopsy of bone marrow of 46 cases of aspiration failure

Histopathological diagnosis	No. of patients	Percentage
Acute leukemia (AL)	19	41.30
Chronic myeloproliferative disorders (CMPD)	13	28.26
Hypoplastic anemia (HA)	07	15.22

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Non-Hodgkin's lymphoma (NHL)	02	4.34
Multiple myeloma (MM)	01	2.17
Reactive marrow (RM)	01	2.17
Metastatic carcinoma (MC) poorly differentiated	01	2.17
Undiagnosed (UD)	02	4.34

Table 3:

Diagnosis	No. of patients	Percentage
Acute Leukemia	19	100
Acute Myeloblastic leukemia (AML)	08	42.10
Acute Lymphoblastic Leukemia (ALL)	06	31.58
Acute Leukemia (undifferentiated) (AL-UD)	05	26.32

[Table 4] shows clinicopathological correlation of 46 cases of haematological disorders in aspiration failure cases. It is evident from the table 4 that the clinical diagnosis of all the cases of Myelofibrosis (n=3), Hypoplastic anaemia (n=5) and lymphoma (n=1) show 100% concordance with histopathological diagnosis. Out of 19 cases of clinically diagnosed AL, histopathological diagnosis was AL in 17 cases (AML-7, ALL-5, AL-UD-5), HA in one and another one as MF. These findings show 89.5 % concordance with clinical diagnosis. Out of 10 cases of clinically CML, histopathological diagnosis was CML in six cases, AML in one and MF in the rest three cases which shows 60 % concordance. One case of clinically diagnosed chronic lymphocytic leukemia (CLL) was histologically lymphoma showing 0% concordance. Among the three cases of clinically diagnosed Kala-azar (KA), histologically one was ALL, one HA and one reactive marrow showing 0% concordance. Out of two clinically diagnosed multiple myeloma, histologically one was MM and one was metastatic carcinoma showing 50% concordance. So it is evident that out of 46 cases, clinical and histopathological diagnoses in 33 cases were similar showing 71.74 % concordance.

Histomorphological patterns in trephine biopsy of mi bone marrow of various haematological and non-Haematological Disorders:

Acute myeloid leukemia: Sections of all of the AML cases (eight patients) revealed hypercellular marrow with increased M: E ratio. The marrow shows predominantly myeloblasts. Erythropoiesis was depressed in seven cases and normal in one. Granulopoiesis was depressed in three cases, active in three and hyperactive in two cases and all revealed shift to the left. Megakaryocytes were absent in four cases, scanty in two and normal in two cases.

Acute lymphoblastic leukemia: Sections of two cases were cellular but others were hypercellular. Majority of the cells were monomorphic consistent with lymphoblasts. All showed increased M: E ratio. Erythropoiesis is depressed in five cases but active in one case. Five showed depressed granulopoiesis but one was active. Five showed depressed granulopoiesis but one was hyperactive. Megakaryocytes were normal in one, scanty in two and nil in three cases.

Myelofibrosis: Sections show hypocellular marrow in six cases, hypercellular in one case but increased fibrosis in all cases. The fibrosis was mostly moderate and diffuse in all the cases. There was depressed haemopoiesis in hypocellular marrow but active haemopoiesis in hypercellular marrow. None of the case revealed any leukemic cell.

Chronic myeloid leukaemia: Aspirated Marrows were hypercellular with increased M: E ratio. This cellularity is due to increase in granulocytes and it's precursors and megakaryocytes. Erythropoiesis was depressed in all. Fibrosis was normal in all cases except one where there was moderately increased reticulin fibrosis confirmed by Gomori's silver impregnation (reticulin stain) of the sections.

Hypoplastic anaemia: Sections of all the cases showed hypocellular marrow. The marrow spaces were occupied predominantly by adipose tissue with scattered lymphocytes and plasma cells. In one case, a few spaces contained dense collections of lymphocytes and plasma cells. Haemopoiesis of all three lineages were depressed.

Non-Hodgkin's lymphoma: Sections show hypercellular marrow with increased M:E ratio in both the cases. In one case, haemopoiesis was depressed but in another, it was active. There was markedly increase number of mature lymphocytes with diffuse and focal distribution.

Reactive marrow: Sections showed normocellular marrow with normal and active haemopoiesis. Increased number of plasma cells, lymphocytes and histiocytes were seen.

Multiple myeloma: The sections show moderate cellular marrow. Marrow spaces contained increased number of plasma cells, some of which appeared immature. These cells were diffusely distributed. In this case, erythropoiesis was depressed but granulopoiesis was active and megakaryocytes were present.

Metastatic carcinoma: Sections revealed, in the marrow spaces, large atypical cells with hyperchromatic nuclei and scanty cytoplasm suggestive of a poorly differentiated metastatic carcinoma.

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Table 4: Distribution of chronic myeloproliferative disorders

Diagnosis	No. of patients	Percentage
Chronic myeloproliferative disorders	13	100
Myelofibrosis (MF)	07	53.85
Chronic myeloid leukemia (CML)	06	46.15

Table 5: Clinicopathological correlation of 46 cases where marrow aspiration failed

C/D		Histopathological Diagnosis								Cncd (%)	
	Total	AL	CML	MF	HA	LYM.	MM	MC	RM	UD	
AL	19	17		01	01						87.5
CML	10	01	06	03							60.0
MF	03			03							100
CLL	01					01					0
HA	05				05						100
KA	03	01			01				01		0
MM	02						01	01			50
LYMP	01					01					100
CDA	02									UD	0
Total	46	19	06	07	07	02	01	01	01	02	

DISCUSSION

The purpose of this study was to see the histomorphological patterns of trephine biopsy of bone marrow in aspiration failure cases. The patients were selected from departments of haematology and medicine of the same institution. In this study, 50 cases of clinically diagnosed haematological and non-haematological diseases were included, where aspiration failure occurred, in four cases, trephine biopsy failure occurred. In the rest 46 cases, adequate material was obtained and their morphological features were studied. Laboratory investigations were also considered in this study.

Out of 46 cases, 19 cases were diagnosed histologically as acute leukemia. This comprised 41.30% of the total. Navone and Colombanol.[6] reported 16% (35 out of 218) acute leukemia in aspiration failure cases. The discordance between two studies may be due to larger sample size of the later.

Out of 19 cases of histologically diagnosed acute leukemia, eight (43.10%) were acute myeloid leukemia, six (31.58%) were acute lymphoblastic leukemia and five cases (26.32%) were acute leukemia (undifferentiated). Categorizing these cases of Al (undifferentiated) into AML or ALL could not be done in our laboratory from the sections of trephine biopsy alone. For this purpose, immunohistochemistry and other tests were not available here. Burkhardt et al.[7] reported 116 cases (88.55%) of acute myeloid leukemia and 15 cases (11.45%) of acute lymphoblastic leukemia out of 131 cases of acute leukemia. To be noted that Burkhardt et al.[7] studied acute leukemia cases in
combined bone marrow aspiration and trephine biopsy samples.

Out of 46 cases, 13 cases (28.26%) were of chronic myeloproliferative disorders. Navone et al.[6]
reported 9.8% cases (44 out of 445) of chronic myeloproliferative disorders in aspiration failure cases. This discordance may be due to its larger sample size. In a study of 850 cases, Burkhardt et al.[8] found 186 cases (21.88%) of chronic myeloproliferative disorders among which CML was in 108 (58.06%) cases and Myelofibrosis in 78 (41.94%) cases. In the present study, among 13 cases of myeloproliferative disorders in aspiration failure cases, 6 cases (46.15%) were diagnosed as chronic myeloid leukemia and 7 cases (53.85%) were diagnose as Myelofibrosis.

In the present study, seven patients out of 46 were histopathologically diagnosed as hypoplastic anaemia. This comprised 15.22% of the total. Burkhardt et al.[8] reported 60 patients of aplastic anaemia out of 441 patients of different diseases. This showed 13.60% of the total.

Two cases (4.34%) were diagnosed histopathologically as non-Hodgkin's lymphoma (NHL). Brunning et al.[9] studied 343 trephine biopsies of lymphoma and other neoplastic diseases and found 50 cases (14.57%) of non-Hodgkin's lymphoma in bone marrow.

Out of 46 cases in this study, histologically. he case (2.17%)was diagnosed as multiple myeloma, one case (2.17%) reactive marrow and one case (2.17%) as metastatic carcinoma (poorly differentiated). Burkhardt et al (1982) reported 10 patients of multiple myeloma out of 8216 patients of different diseases. This showed 0. 12% of the total. Navone et al (1984) reported 4.8% multiple myeloma in their study which was close to the present study. They showed 8.8% non-Hodgkin's and Hodgkin's lymphomas and 13.5% metastatic carcinomas. But Brunning et al9 reported out of 343 cases of lymphoma and other neoplastic diseases, 10 cases of non-hematologic malignancy metastatic to bone marrow which comprised 2.91% of the total which showed concordance with present study.

Out of 46 cases in the present study, two cases with adequate material could not be diagnosed due to poor preparation of the biopsy material.

CONCLUSION

A total of 50 cases of aspiration failure cases were trephined to obtain bone marrow from posterior iliac crest, out of which four cases, trephine biopsy of bone

ISSN (O): 2687-5365; ISSN (P): 2753-6556

marrow failure occurred in which no marrow element were found. The 46 cases with adequate biopsy material revealed histologically 41.30% acute leukemia, 28.26% chronic myeloproliferative disorders, 15.22% hypoplastic marrow, 4.34% non-Hodgkin's lymphoma and 2.17% each of multiple myeloma, reactive marrow and metastatic carcinoma. Out of 46 cases, diagnosis could not be done in two cases (4.34%) due to poor preparation of biopsy material. In a similar study, Navone et al (1984) reported 16% acute leukaemia, 9.8% chronic myeloproliferative disorders, 8.8% malignant lymphoma and Hodgkin's diseases, 4.8 % myelomas and 13.5% metastatic carcinomas in trephine biopsy of bone marrow aspiration failure cases. The discordance between these two studies may be due to difference in their sample size.

In the present study, two cases remained undiagnosed and five cases of acute leukemia could not be subcategorized into AML or ALL. It appears that as only H&E and reticulin stains were used in this study, those cases could not be diagnosed or subcategorized due to lack of other facilities. So it is recommended that facilities for plastic embedding. Immunocytochemistry and use of enzyme and immunophenotyping should be developed in the department and used whenever necessary and study of larger sample size is required for better evaluation.

Conflicts of interest: There is no conflict of interest.
Financial support: Nil

REFERENCES

1. Jamshidi K, Swaim WR : Bone marrow biopsy with unaltered architecture. A new biopsy device. J Lab Clin Med 1971, 77: 335-342.
2. Grann C, Pool JL, Mayer K: Comparative study of bone marrow aspiration and biopsy in patients with neoplastic disease. Cancer 1966, 19:1898-1900.
3. Sing G, Krause JR, Bretfeld V : Bone marrow examination for metastatic tumou - Aspirate and biopsy. Cancer 1977, 40:2317-2321.
4. Brynes RK, McKenna RW.. Sundberg RD : Bone marrow aspiration and trephine biopsy - an approach to a thorough study. Am J Clin Pathol 1978, 70:753-759.
5. Lee GR, Bithell TC, Foster J, Athens JW, Lukens JN, Eds : Wintrobe's Clinical Haematology, 9th edn, vol-1 pp-64-74, Lea and Febriger, Philadelphia, 1993.
6. Navone R and Colombano MT : Histopathological trephine biopsy findings in cases of "dry tap" bone marrow aspirations. Appl Pathol 1984, 2:264-271.
7. Burkhardt R, Bartle R, Jager K, Frisch B, Kettner G, Mahl G, Sunds M : Working classification of chronic myeloproliferative disorders based on histological. Haematological and clinical findings. J Clin Pathol 1986, 39:237-252.
8. Burkhardt R, Frisch B, Bartle R : Bone marrow biopsy in haematological disorders. J Clin Pathol 1982, 35:257-284.
9. Brunning RD, Mckenna RW, Eds: Tumours of the bone marrow. Atlas of Tumour Pathology, Third Series, Fascicle Armed Forces Institute of Pathology. Washington D.C. 1994.

ISSN (O): 2687-5365; ISSN (P): 2753-6556