Original Article

Bone Marrow Examination: A Useful Aid in Early Diagnosis of PUO in Children

Arijit Majumdar, Soumali Biswas[1], Angshuman Jana[2]

Department of Pathology, DMGMC & H, Purulia, [1]Department of Anatomy, Institute of Post-Graduate Medical Education and Research, Kolkata, [2]Department of Microbiology, BSMC, Bankura, West Bengal, India

Abstract

Background: Pyrexia of unknown origin (PUO) is a common diagnostic dilemma that needs a number of diagnostic modalities to arrive at a diagnosis. Bone marrow examination is one of the common tests implicated in the diagnosis in combination with others. Authors, in this study, have attempted to explore the role of bone marrow morphological study in determining the various causes of PUO in children. **Materials and Methods:** A retrospective study was conducted at Dr. B. C. Roy PGIPS, Kolkata, West Bengal over the period of 2 years. Bone marrow aspiration was performed and evaluated morphologically in 100 patients. Marrow trephine biopsy was also performed in some cases, undiagnosed in aspiration smears, to reach at a conclusion. **Results:** Besides reactive hyperplasia (25%), other frequent outcomes were acute lymphoblastic leukemia in 15 (15%), marked hemophagocytosis in 15 (15%), and visceral leishmaniasis in 5 (5%) patients. **Conclusion:** Early diagnosis of PUO can be aided by the morphologic examination of bone marrow. Bone marrow biopsy is a complementary and in some respects, better diagnostic tool than aspiration in patients with PUO. This study will help to know the current spectrum of diseases causing PUO in children in this region.

Keywords: Bone marrow examination, hematological, malignancy, pyrexia of unknown origin

Introduction

Petersdorf and Beeson[1] defined pyrexia of unknown origin (PUO) as "a temperature of 38.3^{0}C (101°F) or greater on several occasions for more than 3 weeks duration and failure to reach a diagnosis despite 1 week of inpatient investigations." Although there is no standard definition of pediatric PUO, fever lasting anywhere from 10 days to 3 weeks is generally accepted as the working definition of PUO in children.[2,3] However, being a challenging medical problem even today PUO calls for many unnecessary over-the-counter laboratory tests and antimicrobial therapies. There is no gold "standard" process to investigate the etiology of PUO.[4] The diagnosis can be unfolded by a critical appraisal of all the available facts. But affordability is a limitation in developing countries like ours.

Bone marrow examination is an important procedure in arriving at a diagnosis for long-duration febrile illness. Bone marrow modifications resulting from infections and systemic diseases can be studied by analysis of marrow morphology. It can impart great influence in the management of patients with undiagnosed fever. There are very few studies regarding the role of bone marrow morphology study in determining the etiology of PUO among children in India, particularly in West Bengal. In this study, an attempt has been made to find out the role of bone marrow study in finding the causes of PUO in children. It can be used as an important early diagnostic tool for various causes of long-standing febrile illness of undiagnosed etiology.

Materials and Methods

A retrospective study was done at the Department of Pathology in a tertiary care hospital over a period of 2 years from January 2019 to December 2020. Patients'

Access this article online

Quick Response Code:

Website: www.medjbabylon.org

DOI: 10.4103/MJBL.MJBL_7_22

Address for correspondence: Dr. Arijit Majumdar, Vill – Duilya, Charaktala, PO – Andul, Howrah 711302, West Bengal, India.
E-mail: arijitmajumdar23@gmail.com

Submission: 11-Jan-2022 **Accepted:** 31-Jan-2022 **Published:** 30-Jun-2022

How to cite this article: Majumdar A, Biswas S, Jana A. Bone marrow examination: A useful aid in early diagnosis of PUO in children. Med J Babylon 2022;19:227-31.

age, sex, clinical history, indication for the procedure, and provisional diagnosis made by the pediatricians were recorded. After routine hematological investigations, bone marrow aspirates were obtained from the posterior superior iliac spine in all patients over 1 yr. Of age and from tibial tuberosity in patients aged below 1 year. Informed consent from the parents was taken in all the cases.

100 patients with PUO underwent bone marrow aspiration as a part of the diagnostic workup of PUO. These cases were reviewed for their clinical data and hematological findings, including detailed morphological features in aspiration smears. Twenty-one patients required trephine biopsy for final diagnosis whereas the rest were diagnosed by bone marrow aspiration alone. Peripheral blood and bone marrow smears were prepared and stained by Leishman stain, while trephine was decalcified and paraffin-embedded blocks were stained with usual hematoxylin and eosin (HandE) stain. Myeloperoxidase (MPO) and periodic acid Schiff (PAS) stain were also used in cases of acute leukemia. In suspected tuberculosis, Zeihl-Neelsen (ZN) stain was performed. Preliminary tests like peripheral smears for malaria, urine examination, liver function tests, Widal test, Mantoux test were performed in all the cases before labeling them as PUO. Cultures for bacterial, fungal, and acid-fast bacilli were performed including blood agar for gram-positive cocci or MacConkey agar for gram-negative, rod-shaped organisms. Blood cultures for mycobacteria were performed with the BACTEC 13A bottle. The biopsy specimens were reviewed for granuloma formation and stained for fungi and acid-fast bacilli, using the Gomori methenamine silver and Fite or Ziehl-Neelsen methods, respectively. PAS stain was also used for demonstration of Fungus. Relevant clinical, radiological, and laboratory findings were also recorded.

The parameters studied were cellularity of marrow particles; status of all series of hematopoietic cells increase in plasma cells, presence, and type of granulomas, and any other associated features seen including hemophagocytosis.

It was a cross-sectional study. The obtained data were collated and results were tabulated. The data were analyzed by measures of central tendency. The institutional ethical committee approved the study [letter no. BCH/ME/PR/1735 dated: 15.01.2019].

Ethical consideration

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the letter number BCH/ME/PR/1735 dated: 15.01.2019 to get this approval.

Results

A total of 100 children were enrolled in this study, including 58 boys (58%) and 42 girls (42%). The mean age was 8.5 ± 5.5 years (range 2 months to 15 years). Age distribution is shown in Table 1.

All the patients revealed a history of prolonged fever ranging in duration from 3 weeks to 3 months, with an average duration of 30 days. Various morphological changes were seen in patients with PUO on bone marrow aspiration which are shown in Table 2.

Various infectious causes were seen in patients with PUO on bone marrow aspiration which are shown in Table 3.

The conditions those are unrelated to PUO but are found incidentally during the investigation are listed in Table 4.

Table 1: Age distribution of patients with PUO (*n* = 100)

Age groups (in years)	No. of cases	Percentage
<1	10	10%
1–5	25	25%
6–10	35	35%
11–15	30	30%

Table 2: Conditions diagnosed by bone marrow morphology (*n* = 100)

Diagnosis	No. of cases	Percentage
Normal	07	7%
Reactive hyperplasia	25	25%
Hemophagocytosis	15	15%
Infections	15	15%
Neoplasia	20	20%
Others	18	18%

Table 3: Infections as a cause of PUO diagnosed by bone marrow morphology (*n* = 15)

Diagnosis	No. of cases	Percentage
Tuberculosis	07	46.7%
Leishmaniasis	05	33.2%
Cytomegalovirus	01	6.7%
Fungal infection	01	6.7%
Malaria	01	6.7%

Table 4: Conditions incidentally found, unrelated to PUO (*n* = 18)

Diagnosis	No. of cases	Percentage
Megaloblastic erythropoiesis	06	33.3%
Micronormoblastic erythropoiesis	04	22.2%
Hypoplastic marrow	05	27.8%
ITP	03	16.7%

Table 5: Number with normal or reactive marrow (n = 40) and the final diagnosis of each case

Diagnosis	No. of cases
Tuberculosis	4
Hemophagocytosis	1
Malaria	1
CMV infection	1
Fungal infection	1
Reactive marrow—no specific cause identified	25
Normal marrow—nospecific cause identified	7

Table 6: Distribution of neoplastic etiologies (n = 20)

Diagnosis	No. of cases	Percentage
Acute lymphoid leukemia (ALL)	15	75%
Acute myeloid leukemia (AML)	1	5%
Myelodysplastic syndrome (MDS)	1	5%
Hodgkin lymphoma (HL)	1	5%
Non-Hodgkin lymphoma (NHL)	2	10%

Initially, we found total 40 cases of normal or reactive hyperplasia of bone marrow. After thorough investigations we were able to find out the specific cause of eight(08) of them which are demonstrated in Table 5.

Out of total 20 cases of neoplasia, majority were of acute lymphoid leukemia (15 cases). Distribution of malignancy is shown in Table 6.

Discussion

Fever in children causes considerable anxiety to their parents and if the fever remains undiagnosed for a prolonged period, it becomes a source of confusion and frustration for the attending physician. This leads to unnecessary and additional over-the-counter laboratory tests and medications (including antimicrobial agents), resulting in an increase in the burden on the patient's family. A well-designed systemic investigation protocol conducted according to local epidemiological information may save time and reduce this unnecessary medical costs and drug-induced complications. PUO resulting from various infectious and systemic diseases is found to incur some morphological changes in the bone marrow morphology.[5] Bone marrow examination thus can clinch an unsuspected diagnosis when other test results are noncontributory or inconclusive during the evaluation process.[6,7] It is found to be a useful investigative tool to diagnose many hematological and nonhematological disorders.[8] Though bone marrow aspiration and trephine biopsy is a painful invasive intervention but the diagnosis made by this can be lifesaving in many patients.[9] In developing countries like ours where patients cannot afford the burden of laboratory expense, bone marrow examination can become the ultimate modality for early diagnosis of PUO.

This study shows 25% of the marrow smears with reactive changes. This is in contrary to a study conducted before where 5% of the cases showed reactive changes.[10] In one of the studies, reactive hyperplasia in children was seen in 12% cases.[11] Reactive hyperplasia is non-specific morphological changes of bone marrow in response to various inflammatory and infective conditions. Severe bacterial infections lead to leukocytosis, mostly granulocytic with or without maturing cells. Toxic granules may be evident in the cytoplasm of the granulocytes.[12]

In our study, 20% cases showed primary malignancies in their bone marrow [Table 6], most common (75%) being Acute lymphoid leukemia (15 cases). non-Hodgkin Lymphoma accounted in 2 cases, AML, MDS, and Hodgkin lymphoma constituted 1 case (5%) each as shown in Table 3. No solid tumors or metastatic tumors were detected in bone marrow examination in our study. This could be due to minimum chance of secondaries in pediatric population. These findings are similar to the study done by Haq *et al.*,[13] where leukemia constituted the commonest malignancy causing PUO. Knokaert *et al.*,[14] in their study, found malignancy as a cause of PUO in 7% cases. In their study, Noor *et al.*[15] showed acute lymphoblastic leukemia as the commonest cause (23.3%) of PUO in children.

We found hemophagocytosis in 15 cases (15%),mostly (10 cases) in children less than 3 years. Familial hemophagocytic lymphohistiocytosis develop early in life; about 70% present at less than 1 year of age.[16]Sharma *et al.*[10] found similar result (haemophagocytosis,14.8%) in their study.

Hassan *et al.*[17] found in their study that the commonest cause of PUO in children are the infectious diseases. Sometimes atypical presentations or inappropriate use of antibiotic prior to the referral may result in delay in the diagnosis. In our study, 15 cases of infectious etiology were detected. Seven cases of tuberculosis (46.7%) were found, of which three were AFB positive. It has to be remembered that the failure to detect acid-fast bacilli in histological sections does not exclude a diagnosis of tuberculosis since organisms are identified in only about 25% of the marrow biopsies in documented disease.[18] In a clinicopathologic analysis of 58 cases by Bodem *et al.*,[19] 4 cases had tuberculosis with granuloma in the bone marrow, but only 2 out of 4 cases showed AFB positively. Similar studies also detected granulomatous disease on biopsies only.[20,21]

Leishmaniasis was detected, in our study, in 05 cases (33.2%). Studies had been carried out showing the

sensitivity of bone marrow being equivalent to splenic aspirates for diagnosing leishmaniasis.[22] Gupta *et al.*[23] found 18 cases from their study showing L.D. bodies in aspirate smears. The trephine biopsy showed an increase in histiocytes with few intracellular L.D. bodies. There was also an associated plasmacytosis found in the marrow. In two patients diagnosed as pure red cell aplasia on aspiration smears, trephine biopsy showed similar features.

Mirdha *et al.*[24] identified malaria in the bone marrow of 8 out of 120 cases with PUO. We found one plasmodium falciparum case. Scanty parasitemia may result in failure of parasite detection on microscopic examination in chronic, low-grade infection. However diagnostic bone marrow examination is often performed when a patient with suspected infection has a persistent fever.[9]

We found 6 and 4 cases of megaloblastic and micronormoblastic erythropoiesis respectively in our study. This was different from the result of the study done by Davidson *et al*.[25] They found it to be in 22% of patients. Davidson related the severity of anemia to the degree and frequency of fever. Noor *et al.*[15] also found 20.3% cases of megaloblastic anemia in their study. However, those studies were done in adult population.

In this study, hypocellular marrow is seen in 5 cases (5%). Bone marrow aspiration alone was not sufficient to point out the cause of hypoplastic marrow. Trephine biopsy was required to come at a diagnosis. In patients with hypoplastic marrow, bacterial and fungal infections were common secondary to neutropenia. This is in keeping with the result obtained by Jandial *et al.*[9]

In our study, we found that 21% of cases needed trephine biopsy as the aspiration study alone was not sufficient for their proper diagnosis. It is a well-known fact that aspiration is better in making out individual cell morphology, whereas biopsy is useful in the study of bone marrow architectural pattern and distribution.[26] Vijayamohanan *et al.* also found similar results in their study.

Conclusion

To sum up, the bone marrow examination provides a rapid diagnosis of a variety of disorders in children like in adults. It is found to be an adjuvant part of investigation of PUO to reach at an etiological diagnosis. This study will help to know the current spectrum of diseases causing PUO in children in this region. The most important cause of PUO in India is still the infection especially tuberculosis, contrary to the decreasing importance of infections as cause of PUO in the western literature.[27] However, our study shows the utility of trephine biopsy as a complementary and necessary aid to marrow aspiration in arriving at an accurate diagnosis, in spite of its disadvantages. The routine performance of a sequential aspiration followed by biopsy in all cases wherever possible is therefore recommended to facilitate a proper diagnostic workup.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Petersdorf RG, Beeson PB. Fever of unexplained origin: Report on 100 cases. Medicine (Baltimore) 1961;40:1-30.
2. Chow A, Robinson JL. Fever of unknown origin in children: A systematic review. World J Pediatr 2011;7:5-10.
3. Mahmoudi S, Mehrazmay A, Salesi M, Mamishi S. Fever of unknown origin: A retrospective study of 95 children in an Iranian referral hospital. Br J Biomed Sci 2014;71:40-2.
4. Ya-Li C, Fang-Liang H, Chung-Ming H, Po-Yen CA. Clinical approach to fever of unknown origin in children. J Microbiol Immunol Infect 2017;50:893-8.
5. Diebold J, Molina T, Camilleri-Broët S, Le Tourneau A, Audouin J. Bone marrow manifestations of infections and systemic diseases observed in bone marrow trephine biopsy review. Histopathology 2000;37:199-211.
6. Bain BJ. Bone marrow aspiration. J Clin Pathol 2001;54:657-63.
7. Bain BJ. Bone marrow trephine biopsy. J Clin Pathol 2001;54:737-42.
8. Riley RS, Hogan TF, Pavot DR, Forysthe R, Massey D, Smith E, *et al*. A pathologist's perspective on bone marrow aspiration and biopsy: I. Performing a bone marrow examination. J Clin Lab Anal 2004;18:70-90.
9. Roopali J, Ritu B, Vinod K. Different causes of pyrexia of unknown origin on bone marrow examination: An institutional experience. J Med Sci Clin Res 2019;7:519-24.
10. Megha S, Nitin G. Role of bone marrow examination in cases of Pyrexia of unknown origin. J Evid Based Med Healthc 2018;5:3558-61.
11. Joshi N, Rajeshwari K, Dubey AP, Singh T, Kaur R. Clinical spectrum of fever of unknown origin among Indian children. Ann Trop Paediatr 2008;28:261-6.
12. Bain BJ, Clark DM, Lampert IA. Bone Marrow Pathology: Infection and Reactive Changes. 2nd ed. Oxford: Blackwell Science; 1996. p. 51-87.
13. Baruch SB, Cannani J, Braunstein R, Perry C, Ben Ezra J, Polliack A, *et al*. Predictive parameters for a diagnostic bone marrow biopsy specimen in the workup of fever of unknown origin. Mayo Clin Proc 2012;87:136-42.
14. Hot A, Jaisson I, Girard C, French M, Durand DV, Rousset H, *et al*. Yield of bone marrow examination in diagnosing the source of fever of unknown origin. Arch Intern Med 2009;169:2018-23.
15. Ayesha N, Muhammad A, Maria S. Frequency of different causes of pyrexia of unknown origin on bone marrow examination in a tertiary care hospital. Pak Armed Forces Med J 2014;64:463-7.
16. Henter JI, Aricò M, Elinder G, Imashuku S, Janka G. Familial hemophagocytic lymphohistiocytosis: Primary hemophagocytic lymphohistiocytosis. Hematol Oncol Clin North Am 1998;12:417-33.
17. Rasha HH, Ashraf EF, Shaimaa MK. Fever of unknown origin in children: A 6 year- experience in a tertiary pediatric Egyptian hospital. Int J Health Sci (Qassim) 2014;8:13-9.
18. Basu D, Saravana R, Purushotham B, Ghotekar LH. Granulomas in bone marrow: A study of fourteen cases. Indian J Pathol Microbiol 2005;48:13-6.

19. Bodem CR, Hamory BH, Taylor HM, Kleopfer L. Granulomatous bone marrow disease: A review of the literature and clinicopathologic analysis of 58 cases. Medicine (Baltimore) 1983;62:372-83.
20. Chandra S, Chandra H. Comparison of bone marrow aspirate cytology, touch imprint cytology and trephine biopsy for bone marrow evaluation. Hematol Rep 2011;3:e22.
21. Jeevan SK, Paul-Tara R, Uppin S, Uppin M. Bone marrow granulomas: A retrospective study of 47 cases (A single center experience). Am J Int Med 2014;2:90-4.
22. da Silva MR, Stewart JM, Costa CH. Sensitivity of bone marrow aspirates in the diagnosis of visceral leishmaniasis. Am J Trop Med Hyg 2005;72:811-4.
23. Ruchika G, Namrata S, Prerna A, Sompal S, Tejinder S. Hematological profile in pyrexia of unknown origin: Role of bone marrow trephine biopsy vis-àvis aspiration. Hematology 2008;13:307-12.
24. Mirdha BR, Samantray JC, Mishra B, Xess I. Bone marrow examination for identifying malaria in fever of unknown origin. J Assoc Physicians India 1999;47:177-9.
25. Davidson S. Clinical picture of pernicious anaemia prior to introduction of liver therapy in 1926 and in Edinburgh subsequent to 1944. Br Med J 1957;1:241-3.
26. Mahajan V, Kaushal V, Thakur S, Kaushik R. A comparative study of bone marrow aspiration and bone marrow biopsy in haematological and non-haematological disorders: An institutional experience. J Indian Acad Clin Med 2013;14:133-5.
27. de Kleijn EM, Vandenbroucke JP, van der Meer JW. Fever of unknown origin (FUO). I A. Prospective multicenter study of 167 patients with FUO, using fixed epidemiologic entry criteria. The Netherlands FUO Study Group. Medicine (Baltimore) 1997;76:392-400.