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ORIGINAL ARTICLE

ETIOLOGICAL EVALUATION IN 120 CASES OF PANCYTOPENIA BY BONE MARROW EXAMINATION

Surya Babu Sunkesula[1], Lingeswara Rao B[2], Tamil Arasi D. S[3]

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ABSTRACT: BACKGROUND: Pancytopenia is a relatively common hematological entity resulting from a variety of disease processes. The present study is carried out to find out the causes of pancytopenia by bone marrow examination. **MATERIALS AND METHODS:** Bone marrow examination is carried out in 120 cases of pancytopenia over a period of 3 years. Age of patients ranged from 2 to 75 years with a mean of 32 years. Most of the patients presented with generalized weakness and fever. Commonest physical finding was pallor followed by splenomegaly. Dimorphic anemia was the predominant blood picture. The commonest marrow finding was megaloblastic erythroid hyperplasia. The commonest cause of pancytopenia was megaloblastic anemia (58.3%) followed by aplastic anemia (18.3%). Others include acute leukemia, myelodysplasia, non-Hodgkin's lymphoma and disseminated tuberculosis. **CONCLUSION:** The present study shows the importance of bone marrow examination in evaluating pancytopenia and the geographical variation in the etiology as megaloblastic anemia is the predominant cause of pancytopenia in Indian subcontinent.
KEYWORDS: pancytopenia, bone marrow, megaloblastic anemia, aplastic anemia.

INTRODUCTION: Pancytopenia is a disorder in which all three major formed elements of blood (red blood cells, white blood cells and platelets) are decreased than normal. It is a relatively common clinicohaematological entity encountered in our day-to-day clinical practice. Manifestations of peripheral pancytopenia are due to a wide variety of disorders which primarily or secondarily affect the bone marrow.[1] The presenting symptoms are usually attributable to anemia, thrombocytopenia and leucopenia. The severity of pancytopenia and the underlying pathology determine the management and prognosis of the patients. The present study has been undertaken to evaluate the various causes of pancytopenia by bone marrow examination.

MATERIALS & METHODS: The present study included 120 cases during a period of 3years, from January 2011 to December 2013 in our hospital. Patients of all age groups and both sexes included. Patient selection was based on clinical features and peripheral blood findings which included hemoglobin, leucocytes and platelets. Inclusion criteria were presence of all 3 of the following: hemoglobin less than 9 g/dL; total leucocytes count (TLC), < 4000/µL: platelet count < 1,00,000/µL. Patients on myelotoxic chemotherapy were excluded.

Two mL of anticoagulated blood was collected and processed through an automated hematology analyzer; and 9 hematological parameters were obtained, which included Hb, RBC count, total leucocyte count, differential count, Platelet count, MCV, MCH, MCHC and PCV. ESR was estimated in all cases by Westergren's method. Peripheral smear was stained by Leishman's stain for all the cases and examined in detail for red cell morphology, platelet count and white cell morphology. Reticulocyte count was done using 1% Brilliant Cresyl blue for supravital staining.

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Bone marrow aspiration and biopsy were done in all cases of pancytopenia. Bone marrow aspiration slides were stained by Giemsa stain and examined in detail. Bone marrow trephine biopsy was done for each case, then, after fixation and partial decalcification, stained with H&E. Reticulin stain was also done on biopsy sections and extent of fibrosis was graded by modified Bauermeister scale.

RESULTS: In this study a total of 120 cases of pancytopenia were studied. They consisted of 72 males and 48 females with a male to female ratio of 1.5:1. The age of the patients ranged from 2 to 75years (mean age 32 years). Out of 120 cases, 38 were pediatric patients. One case of familial bone marrow failure syndrome was observed.

The commonest clinical presentation was generalized weakness (100%); other main symptoms were dyspnea (25%), fever (38.3%) and weight loss (12%). Pallor was noted in all cases (100%). Splenomegaly was noted in cases of megaloblastic anemia, followed by acute leukemia. Bony tenderness and Lymphadenopathy were noted in leukemia patients.

Peripheral smear showed dimorphic anemia followed by macrocytic anemia in majority of cases. Leucopenia and thrombocytopenia were seen in all cases. Megaloblastic anemia was observed in 70 patients (38 males and 32 females) with their age ranging from 8 to 75years. Three patients had neurological deficits in the form of sensory ataxia. Peripheral smear showed macrocytes, macroovolocytes, increased MCV and hyperlobated neutrophils. Bone marrow aspiration showed megaloblastic erythroid hyperplasia (Fig.1). Giant metamyelocytes and giant band forms were seen in myeloid series.

Since B_{12} and folate levels could not be estimated as a routine, both folic acid and parental hydroxycobalamine therapies were administered to all, and they showed complete hematological response. The hematological response was estimated by reticulocyte count which showed increased count after the initiation of therapy.

Aplastic anemia was seen in 15 males and 7 females; their age ranged from 11 to 75years with a mean age range of 32years. Out of 22 cases with bone marrow hypoplasia, cause was not known in 19 cases. One case was a familial bone marrow failure syndrome which was later diagnosed as Fanconi anemia by chromosomal breakage analysis. Clinically, this patient had café aü lait spots, skeletal abnormalities and stunted growth. Another patient was later diagnosed as a case of PNH detected by gel card test for showing negativity for DAF (CD 55) and MIRL (CD 59). One patient gave history of treatment with Carbamazepine for epilepsy.

Bone marrow aspiration showed reduced cellularity with increased stromal elements and fat spaces (Fig.2). Myeloid and erythroid series and megakaryocytes were reduced. There is a relative lymphoplasmacytosis. Perls stains showed increased iron stores. Trephine biopsy showed increased fatty infiltration and reduced cellularity in the marrow space.

We diagnosed 11 cases of acute leukemia on bone marrow examination. Their age ranged from 2 to 45years. Of them, 6 cases were acute myelogenous leukemia. Out of 6 cases, 3 were APML one was AML-M2, one was AML – M4/M5 and in one case there was persistence of disease in a diagnosed and treated case of AML. Four cases were ALL and all were children under the age of 10years. One child with ALL showed dry tap on BMA. The biopsy imprints and trephine biopsy sections showed blast prominence. One was case of CML on imatinib therapy having myeloid blast prominence on bone marrow examination.

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Clinical presentation, morphology of blasts and special stains with SBB and PAS were helpful in differentiating myeloid and lymphoid blasts (Fig. 3).

Two case of myelofibrosis were noted. There was a dry tap while marrow aspiration. Peripheral smear showed leucopenia with tear drop erythrocytes and nucleated RBC. Massive Splenomegaly noted. One was a known and treated case of CML with marrow fibrosis. Reticulin stains in both cases showed grade 4 condensation (Fig. 4).

Myelodysplastic syndrome was detected in 4 patients. In 3 patients, bone marrow aspiration showed increased cellularity with dyserythropoiesis, dysmyelopoiesis and dysmegakaryopoiesis. One case showed marrow hypoplasia with 8% blasts on BMA which turned out to be hypoplastic MDS on trephine biopsy. All showed abnormal localization of immature precursors (ALIP) on trephine sections.

Four cases showed marrow involvement by non-Hodgkin's lymphoma (Fig.5). Of them, one was a retroviral positive case. Another was a four year old boy with Burkit's lymphoma.

Pancytopenia due to disseminated tuberculosis in bone marrow was seen in 3 cases. One was a diagnosed and treated case of tuberculosis and in the remaining two cases, the primary diagnosis was made on trephine biopsy sections. Epithelioid granulomas were detected in trephine biopsy sections (Fig. 6).

Malarial infestation was seen in 2 patients. Peripheral smear showed Plasmodium falciparum gametocytes. Patients had massive splenomegaly with pancytopenia. Bone marrow showed erythroid hyperplasia. Patients recovered after antimalarial treatment.

One was a known case of ovarian malignancy with metastasis to bone marrow.

We encountered a case of storage disorder in a 12 year old male patient presented with fever, hepatosplenomegaly and pancytopenia. Bone marrow showed numerous large foamy histiocytes with crumpled tissue paper appearance. PAS done on the slides was negative. The diagnosis was considered as Niemenn–Pick's disease.

Etiology of pancytopenia	Number of cases (Total: 120 cases)	Percentage (%)
1. Megaloblastic anemia	70	58.33
2. Aplastic anemia	22	18.33
3. Acute leukemia	11	09.16
4. Myelofibrosis	02	01.66
5. Disseminated tuberculosis	03	02.50
6. Myelodysplastic syndrome	04	03.33
7. Non-Hodgkin's lymphoma	04	03.33
8. Malaria with splenomegaly	02	01.66
9. Metastatic carcinoma	01	0.83
10. Storage disorder	01	0.83

Table 1: showing distribution of various causes of pancytopenia in the present study

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Figure 1: shows bone marrow aspiration (BMA) cytology showing megaloblastic erythroid hyperplasia (Giemsa, ×100). Inset shows morphology of megakaryocytes (Giemsa, ×400).

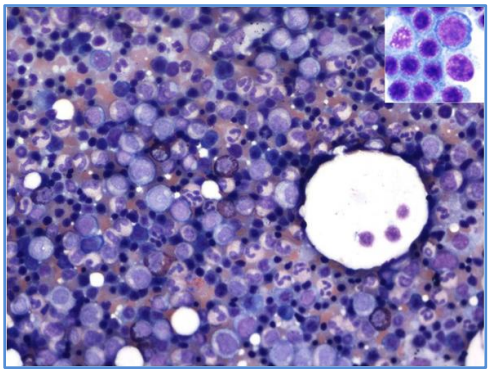

Figure 1

Figure 2: shows BMA cytology showing marrow hypoplasia with relatively increased fat spaces and stromal fragments (Giemsa, ×100). Inset shows trephine biopsy having bony trabeculae with intervening marrow showing reduced cellularity and replacement by fat (H&E, ×100).

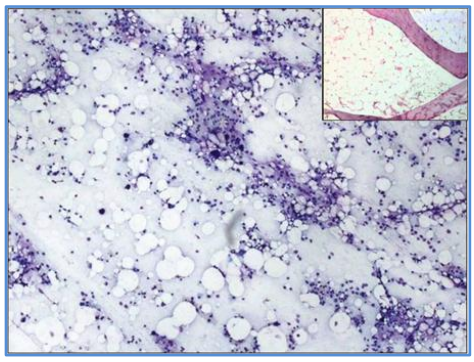

Figure 2

Figure 3: showing BMA cytology in a case of ALL showing blasts accounting for >90% of the cellularity (Giemsa, ×400). Inset shows blasts showing magenta colored block positivity with Periodic acid Schiff stain (PAS, ×1000).

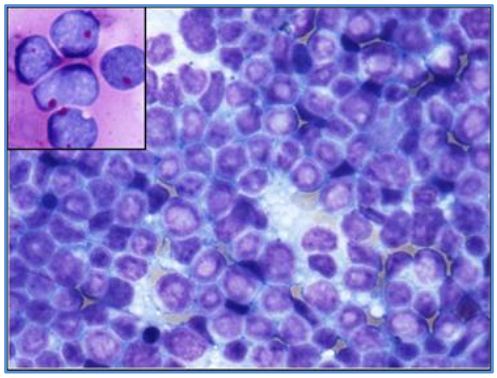

Figure 3

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Figure 4: showing trephine biopsy from a patient of myelofibrosis showing grade 4 condensation (reticulin stain, ×100).

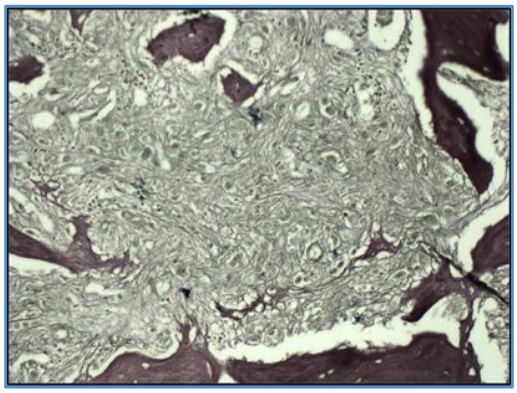

Figure 4

Figure 5: shows trephine biopsy showing involvement by lymphoma (H&E, ×100).

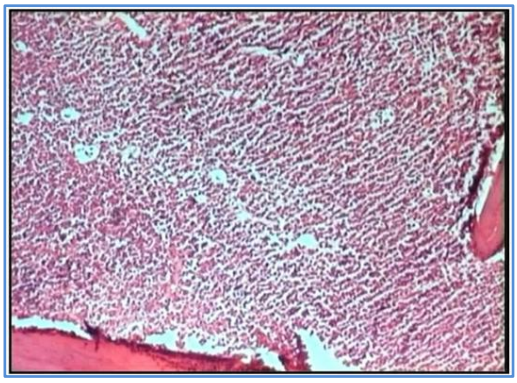

Figure 5

Figure 6: shows trephine biopsy showing epithelioid granuloma with giant cells in a case of tuberculosis (H&E, ×100).

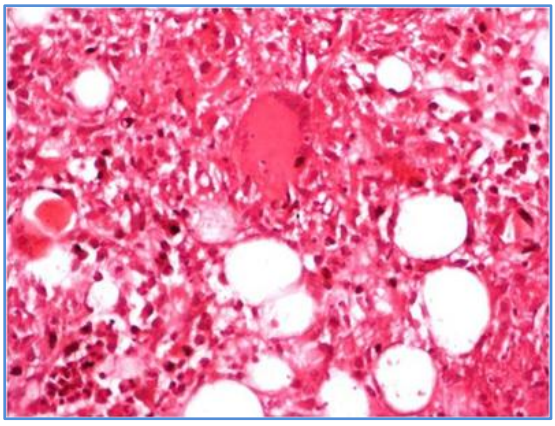

Figure 6

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DISCUSSION: A total of 120 cases of pancytopenia were studied. Age, gender-wise incidence, presenting complaints, peripheral blood picture, bone marrow examination and different causes of pancytopenia were studied in all cases, and observations were compared with those in studies published in the literature.

The age of patients ranged from 2 to 75years (mean age 32years) with a male to female ratio of 1.5:1. The commonest clinical presentation was generalized weakness and other main symptoms were dyspnoea, fever and weight loss. Pallor was the most common physical finding followed by splenomegaly and hepatomegaly. The clinical features were comparable to other studies.[2]

The commonest cause of pancytopenia, reported from various studies throughout the world has been aplastic anemia.[3] This is in sharp contrast with the results of various Indian studies where the commonest cause of pancytopenia is megaloblastic anemia.[1,4] Results observed in the present study were also similar to those Indian studies. This seems to reflect the higher prevalence of nutritional anemias in Indian subjects. It is a rapidly correctable disorder and should be promptly notified.[1] Hypersegmented neutrophils were noted in 60% compared to 84.9% of cases in Tilak V *et al* in megaloblastic anemia.[4]

Although bone marrow aspiration study is uncommon in a suspected megaloblastic anemia, if the diagnosis does not appear straight forward or if the patient requires urgent treatment and hematological assays not available, bone marrow aspiration is indicated.

As facilities for estimating folic acid and vitamin B_{12} levels are not routinely available in most centers of India, the exact deficiency is usually not identified.[4]

Incidence of aplastic anemia varies from 10-52% among pancytopenic patients.[1,4] Its incidence in the present study is 18.3%. The incidence of aplastic anemia quoted from the west is much higher than that observed by us. This increased incidence may be related to environmental factor such as increased exposure to toxic chemicals.

Myelodysplastic syndrome was detected in 3.3% compared to 2% by Khunger *et al* and others.[1,5,6] One case showed marrow hypoplasia with 8% blasts on BMA which turned out to be MDS on trephine biopsy. All showed abnormal localization of immature precursors (ALIP) on trephine sections. Hypoplastic MDS is a differential diagnosis of aplastic anemia.

We encountered acute leukemia in 9.2% of patients of pancytopenia compared to 5% by Khunger *et al* and 12% by Kumar *et al*.[2,4] Myelofibrosis was seen in 1.6% of our cases of pancytopenia. Literature also reveals similar incidence.[1,7]

Disseminated tuberculosis related to pancytopenia was noted in 2.5% of our cases. Basu *et al*, Yadav *et al* and Singh *et al* have also reported various cases of pancytopenia in disseminated TB in India, and it is necessary to be aware of its manifestations as pancytopenia.[8,9,10]

Malaria related hypersplenism was the cause of pancytopenia in 1.6% of our patients as similar to other studies.[11,12] Pancytopenia related to non-Hodgkin's lymphoma was noted in 3.3% of our patients similar to other studies.[13] As in our study, myelophthisic pancytopenia can also occur due to metastatic carcinomas and storage disorders.[14,15]

To summarize, pancytopenia is a common hematological problem encountered in day-to-day practice. In contrast to western literature, megaloblastic anemia is the most common cause of pancytopenia in India and being a rapidly correctable nutritional disorder, if this is set right, the number of pancytopenia cases reported in hospitals will no doubt get drastically reduced.

REFERENCES:

1. Khunger JM, Arulselvi S, Sharma U, Ranga S, Talib VH. Pancytopenia--a clinic haematological study of 200 cases. Indian J Pathol Microbiol. 2002Jul; 45 (3): 375-9. Review. PubMed PMID: 12785191.
2. Kumar R, Kalra SP, Kumar H, Anand AC, Madan H. Pancytopenia--a six year study. J Assoc Physicians India. 2001 Nov; 49: 1078-81. PubMed PMID: 11868860.
3. Issaragrisil S, Chansung K, Kaufman DW, Sirijirachai J, Thamprasit T, Young NS. Aplastic anemia in rural Thailand: its association with grain farming and agricultural pesticide exposure. Aplastic Anemia Study Group. Am J Public Health. 1997 Sep; 87 (9): 1551-4. PubMed PMID: 9314815; PubMed Central PMCID: PMC1380989.
4. Tilak V, Jain R. Pancytopenia--a clinico-hematologic analysis of 77 cases. Indian J Pathol Microbiol. 1999 Oct; 42 (4): 399-404. PubMed PMID: 11127368.
5. Albitar M, Manshouri T, Shen Y, Liu D, Beran M, Kantarjian HM, Rogers A, Jilani I, Lin CW, Pierce S, Freireich EJ, Estey EH. Myelodysplastic syndrome is not merely "preleukemia". Blood. 2002 Aug 1; 100 (3): 791-8. PubMed PMID: 12130488.
6. Shimazaki K, Ohshima K, Suzumiya J, Kawasaki C, Kikuchi M. Apoptosis and prognostic factors in myelodysplastic syndromes. Leuk Lymphoma. 2002 Feb; 43 (2): 257-60. Review. Pub Med PMID: 11999555.
7. Kiss E, Gál I, Simkovics E, Kiss A, Bányai A, Szakáll S, Szegedi G. Myelofibrosis in systemic lupus erythematosus. Leuk Lymphoma. 2000 Nov; 39 (5-6): 661-5. PubMed PMID: 11342352.
8. Basu S, Mohan H, Malhotra H. Pancytopenia due to hemophagocytic syndrome as the presenting manifestation of tuberculosis. J Assoc Physicians India. 2000 Aug; 48 (8): 845-6. PubMed PMID: 11273486.
9. Yadav TP, Mishra S, Sachdeva KJ, Gupta VK, Siddhu KK, Bakshi G, Yadav RB. Pancytopenia in disseminated tuberculosis. Indian Pediatr. 1996 Jul; 33 (7): 597-9.PubMed PMID: 8979574.
10. Singh KJ, Ahluwalia G, Sharma SK, Saxena R, Chaudhary VP, Anant M. Significance of haematological manifestations in patients with tuberculosis. J Assoc Physicians India. 2001 Aug; 49: 788, 790-4. PubMed PMID: 11837465.
11. Latger-Cannard V, Bibes B, Dao A, Fohlen-Walter A, Buisine J, Rabaud C, May T, Marchand-Arvier M, Lesesve JF, Lecompte T. [Malaria-related cytopenia]. Ann Biol Clin (Paris). 2002 Mar-Apr; 60 (2): 213-6. French. PubMed PMID: 11937447.
12. Aouba A, Noguera ME, Clauvel JP, Quint L. Haemophagocytic syndrome associated with plasmodium vivax infection. Br J Haematol. 2000 Mar; 108 (4): 832-3. PubMed PMID: 10792290.
13. Keisu M, Ost A. Diagnosis in patients with severe pancytopenia suspected of having aplastic anaemia. Eur J Haematol 1990; 45: 11-4.
14. Makoni SN, Laber DA. Clinical spectrum of myelophthisis in cancer patients. Am J Hematol. 2004 May; 76 (1): 92-3. PubMed PMID: 15114608.
15. Gayathri BN, Rao KS. Pancytopenia: a clinico hematological study. J Lab Physicians. 2011 Jan; 3 (1): 15-20. doi: 10.4103/0974-2727.78555. PubMed PMID: 21701657; PubMed Central PMCID: PMC3118050.

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AUTHORS:

1. Surya Babu Sunkesula
2. Lingeswara Rao B.
3. Tamil Arasi D. S.

PARTICULARS OF CONTRIBUTORS:

1. Associate Professor, Department of Pathology, Kurnool Medical College, Kurnool, Andhra Pradesh.
2. Senior Resident, Department of Pathology, Kurnool Medical College, Kurnool, Andhra Pradesh.
3. Professor & HOD, Department of Pathology, Kurnool Medical College, Kurnool, Andhra Pradesh.

NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Lingeswara Rao B,
Senior Resident,
Department of Pathology,
Kurnool Medical College,
Kurnool-518002,
Andhra Pradesh.
Email: eswar.bathula@gmail.com

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