Original Article

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To Study the Role of Bone Marrow Aspiration in Etiological categorization of Pediatric Pancytopenia

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Abstract

Background: Pancytopenia is a Clinico-hematological entity, characterized by a triad of Anemia, leucopenia, and thrombocytopenia. The criterion for defining Pancytopenia is HGB < 9 g/dl: ANC < 1.5 x 109/L and platelet count <100 x 109/L. The main objective of this study is to categorize pediatric pancytopenia's on etiological basis and to study the age wise incidence.

Methodology and Results: This is a 5-year study (2016-2021) conducted in tertiary care pediatric referral hospital. All data were expressed as Mean +/ - SD and p value <0.05 is taken as statistically significant. A total of 500 bone marrow smears of pancytopenic patients were studied, of which 60% were boys & 40% girls, 64% constituted Malignant diseases and 36% were benign. Incidence wise, ALL is the most common and pancytopenia being 2nd and together comprises 64% of total cases. Some rare cases like AML –M6 in 2 months old, Chediak higashi syndrome and Familial Hemophagocytic syndrome were noted. Flow cytometry and Molecular genetics were done were ever required.

Conclusion: Bone Marrow Aspiration is a reliable and sensitive test which can be used in investigation of Pediatric pancytopenia's and if used meticulously with other routine hematological tests can give a high diagnostic accuracy. ALL is most common in our study (other studies showed Aplastic anemia), AML-M6 in 2 months, LD bodies in bone marrow, HPS were amongst the rare cases

Keywords: Leukemia, Pancytopenia, Megaloblastic Anemia, AML, ALL

Introduction

Pancytopenia is a Clinico-hematological entity, characterized by a triad of Anemia, leucopenia, and thrombocytopenia. The criterion for defining Pancytopenia is Hemoglobin (HGB) <9 g/dl; total leukocyte count (TLC) < 4.0 x 109/L or absolute neutrophil count (ANC) is < 1.5 x 109/L and platelet count <100 x 109/L. [1]

Pancytopenia in pediatric age group [2] can be broadly categorized in to

- Malignant Hemato-lymphoid diseases
- Benign conditions like Inherited bone marrow failure syndromes
- Infections
- Drugs /Iatrogenic

The underlying mechanism of pancytopenia are reduction in hematopoietic stem cell production, marrow replacement by abnormal cells (leukemia, lymphoma, storage disorders), suppression of marrow growth and differentiation (Aplastic anemia, infections), ineffective hematopoiesis, defective cell formation, antibody-mediated sequestration and destruction of cells by mononuclear phagocytic system. It can be secondary to Drugs, chemotherapy, radiotherapy, viral and parasitic infections.[3] One of the important causes of impaired hematopoiesis in pediatric age group is congenital TORCH infections (toxoplasmosis, rubella, cytomegalovirus, herpes simplex, and others), hepatitis C, HIV, Helicobacter pylori, and leishmaniasis. [1,2]

Statement of Problem: Pediatric Pancytopenia's often poses diagnostic challenges and early rapid diagnosis is often required for patient management and surveillance.[3] Accurate diagnosis is necessary for correct treatment and erroneous diagnosis can lead to gross mortality and morbidity of patients.[1]

Significance of the Study: Bone Marrow Aspiration is a reliable and sensitive test which can be used in investigation

of Pediatric pancytopenia's and if used meticulously with other routine hematological tests can give a high diagnostic accuracy.[3]

Objectives: To study the role of Bone Marrow Aspiration in etiological categorization of Pediatric Pancytopenia's. To study Age and gender wise distribution of pancytopenia's in children. To study etiologic spectrum of various diseases with varying grades of pancytopenia.

Material and methods

This is a 5-year study conducted between 2016 to 2021 in tertiary care pediatric referral hospital & teaching centre. Ethical Approval and consent were obtained from Institutional review committee, General director of Health affairs in Madinah.

Inclusion Criteria: Pediatric patients from birth to 16 years of Age were included. The local policy of the hospital identifies children up to 16 years of age as pediatric. Criteria of Pancytopenia - Hb < 9g/L, WBC< 4.0x109/L or ANC <1.5X109/L, Platelet count < 100x109/L

Exclusion Criteria: Children > 16 yrs. old. History of Drugs, Chemotherapy, Radiotherapy

In all Patients detailed medical history, CBC, Retic %, Peripheral blood smear, coagulation profile, routine biochemical investigations, ELISA, LFT, Renal function tests, thyroid profiles, Ultrasonography, Auto-immune panels etc. Bone Marrow Aspiration was performed under all aseptic precautions following the standard procedure. Routine CBC was done in Advia, Sysmex n 2000i Auto-analysers. Blood and Bone Marrow smears stained with wright –Giemsa stain following the standard protocol. Flow Cytometry is done wherever necessary.

Statistical analysis: All the data were expressed as Mean +/ - SD and p value <0.05 is taken as statistically significant.

Result

A total of 500 cases were studied of which 60% were boys and 40% girls.

Table 1: Sex wise Incidence

Gender	Boys	Girls
BMA = 500	300 (60%)	200 (40%)

Of the total 500 cases, 36% were Benign and 64% constituted Malignancy (Table-1)

In Benign category, Aplastic Anemia is the most common cause comprising 55% of total benign cases, Infections (20%), Megaloblastic Anemia (11%), Hemophagocytic syndrome (08%), CDA (2.7%) and few rare cases of CHS, secondary Myelofibrosis.

Fanconi's Anemia & DBS comprises 40% of Inherited Aplastic Anemia and Idiopathic Acquired Aplastic Anemia constituted the remainder 60% of cases

Viral etiology is the most common cause in the infective category comprising 41% of total infective causes, and EBV constituted 73% of viral causes. There were 10 cases of Bacterial infections, 6 cases of malaria, 3 cases of LD, one case each of Candida and Histoplasmosis.

Of the 320 Malignant cases, ALL constituted 220 cases (68.7%), AML (25%), Metastasis (1.8%), MDS (1.5%), JMML (1.2%) and 03 cases of Biphenotypic Leukemia.

B-ALL comprised 60% and T-ALL 7% of all malignant cases. AML-M4 & M5 constituted 62% of all AML cases (Table 2).

Majority of ALL cases occurred in < 5years age group (68%). 60% of Fanconi Anemia cases occurred in 5-10 years age group (Table 3).

Figure 1 is from a case of Hemophagocytic /HLH syndrome showing Hemophagocytes engulfing erythroid cells, Figure 2 shows Parvovirus Inclusion in Pro-Erythroblast. Figure 3 is showing purplish Intracytoplasmic Granules in Chediak Higashi Syndrome. Figure 4 is of rare Schizont stage of plasmodium falciparum. Figure 5 & 6 shows binucleated and multinucleated erythroblasts in Congenital Dyserythropoietic Anemia Type 1 & 2 respectively. Leishmania Donovani (LD) body is highlighted in B.M. biopsy in macrophages in figure 7, a rare Juvenile myelo-monocytic leukemia with Monoblasts, Promonocytes, Myeloblasts are noted in Figure 8. Figure 9 & 10, highlights Infections like fungus (candida) & Histoplasmosis respectively, Figure 11 & 12 shows Pawn ball & Mononuclear Megakaryocytes in MDS.

AML showing Myeloblasts and Megakaryoblast in Fig 13 & 14 respectively. Figure 15 & 16 shows AML-M4 with myelomonocytic differentiation, Figure 17 is a rare case of AML –M6 in 2 months old infant showing Trinucleate Erythroblasts, Figure 18 is B-ALL case with Lymphoblasts showing cleaved nuclei. Figure 19 shows Metastatic Neuroblastoma cells with Rossette formation & Figure 20 shows Lymphopblasts from T-ALL case.

Flow cytometry images 21A, B, C, D, E shows Strong +ve

Table 2: Etiological categorization of pancytopenia in BMA

Benign (180) {36%}	Malignant (320) {64%}
1. Aplastic Anemia (AA) 100 (55%) • Inherited AA • Fanconi A - 30(16%) • DBS – 08 (04%) • Pearson's – 02 (01%) • Acquired AA • Idiopathic AAA (33%)	1. ALL – 220 (68.7%) • B Cell ALL 195 (60%) • T cell ALL 25 (7.8%)
2. Infection – 36 (20%) • Parasite – Malaria - 06, LD- 03 • Fungus – Candida -01, HP - 01 • Bacterial – 10 (05%) • Viral – 15 (8.3%) o EBV – 11(6.1%) o CMV – 2 (01%) o Parvo Virus -2 (01%)	2. AML – 82 (25%) • M2 – 08 (2.5%) • M3 – 12 (3.7%) • M4 – 33 (10%) • M5 – 18 (5.6%) • M6 – 04 (1.2%) • M7 – 07 (2.1%)
3. Megaloblastic Anemia - 20 (11%)	3. Biphenotypic Leukemia 03 (0.9%)
4. Rare causes • HPS - 15(8.3%) {Familial (05) / Secondary (10) • CDA – 05 (2.7%) {CDA II – 3) (CDA I & III – 01) • CHS – 03(1.6%) • MF (secondary) – 01(0.5%)	4. Rare Malignancies • JMML – 04 (1.2%) • MDS - 05 (1.5%) • Metastasis - 06 (1.8%)
FA – Fanconi anemia, DBS- Diamond **Blackfan** syndrome, AA- Aplastic Anemia, CHS –Chediak higashi syndrome, MA- Megaloblastic anemia, MDS- Myelodysplastic syndrome, HPS – Hemophagocytic syndrome, JMML – Juvenile Myelo-Monocytic **Leukaemia**	

for CD5, CD34, CD45 markers and -ve for CD19, CD22 in T-ALL case. Flow cytometry image 22A & B is from AML +Ve for CD117, MPO)

Discussion

Pancytopenia is a Clinico-hematological entity, characterized by a triad of Anemia, leucopenia and thrombocytopenia.[1]

Aplastic anaemia is a rare and heterogeneous disorder. It is defined as pancytopenia with a hypocellular bone marrow in the absence of an abnormal infiltrate or marrow fibrosis.

The diagnostic criteria proposed by camitta et al 1975 is Hb < 10g/L, Platelet count < 50 x 109/L, ANC < 1.5 x 109/L. The majority (70-80%) of cases are idiopathic (Marsh et al, 2009) and the remainder mainly consist of inherited bone marrow failure syndrome. The incidence is 2-3 per million per year in Europe, but higher in East Asia (Montane et al, 2008).[2,3]

Various studies done by Kumar [4] et al, Khunger [5]et al, Tilak [6] et al and Khodke [7] et al concluded that megaloblastic anemia is the commonest cause of pancytopenia followed by aplastic anemia, kalaazar and other causes. However studies conducted by Tariq8 et al concluded that aplastic anemia was the most commonest cause. (Table 4).

Naseem et al [9] conducted a study on 175 children with pancytopenia, with male:female ratio of 3.2:1; 78 cases were classified as non-malignant, 37 as malignant, 24 as non-specific and 36 as having iatrogenic/therapy induced pancytopenia. (Table 4)

Shazia menon [10] et al studied the etiological spectrum of pancytopenia in children based on the bone marrow examination. They revealed that the common cause of pancytopenia was aplastic anemia (23.9%), followed by megaloblastic anemia (13.04%), leukemia (13.05%), enteric fever (10.8%), malaria (8.69%) and sepsis (8.69%).

Gunvanti B. Rathod [11] et al did clinico-hematological analysis of pancytopenia in Pediatric patients and stated that

megaloblastic anemia 26.5% was the most common cause of pancytopenia followed by aplastic anemia 20.0%, leukemia 17.5%, idiopathic thrombocytopenic purpura 10%, iron deficiency anemia 9.5%, anemia of chronic disorder 1.5% and finally malaria 3.5%.

Table 3: Age wise etiological categorization of pancytopenia

	< 5 years	5-10 years	>10 years
Benign			
Fanconi Anemia (30)	**10**	**18** (60%)	**02**
DBS	**06** (75%)	**02**	---
Idiopathic AAA	**10**	**35** (58%)	**15**
Infections	**23** (63%)	**10**	**03**
Megaloblastic Anemia	**10**	**07**	**03**
HPS	**08**	**05**	**02**
CHS	**03**	---	---
CDA	**05**	---	---
Malignant			
ALL	**150** (68%)	**60** (27%)	**10** (4.5%)
AML	**15** (18%)	**40** (48%)	**27** (32%)

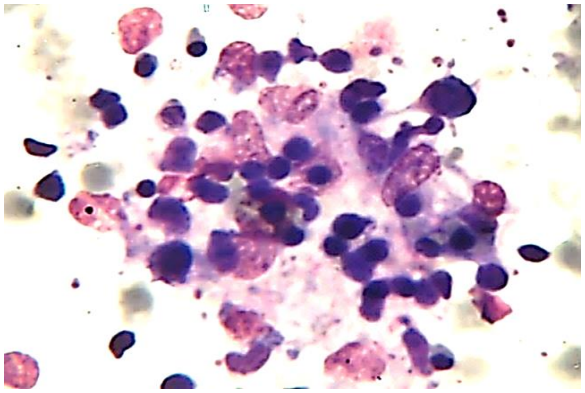

Figure 1: HPS - Hemophagocytes engulfing erythroid cells

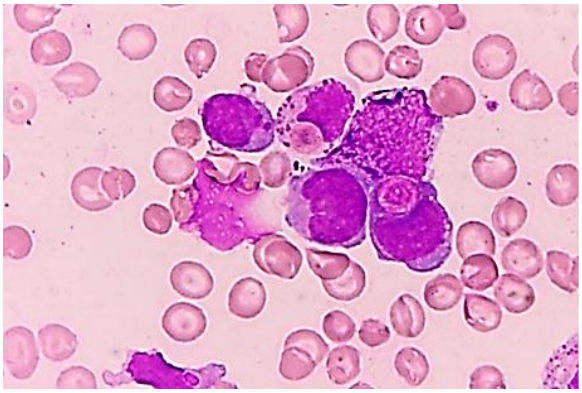

Figure 3: CHS (Purplish Intracytoplasmic Granules)

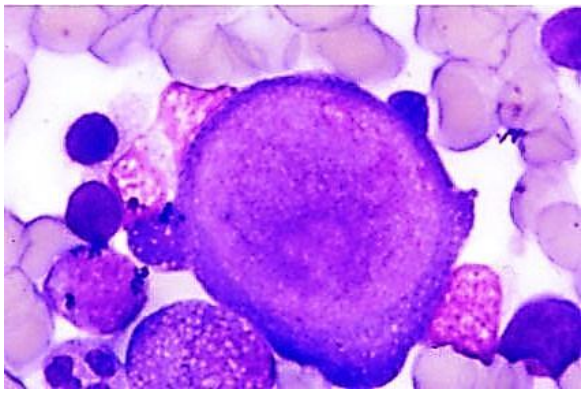

Figure 2: Parvovirus Inclusion in Pro-Erythroblast

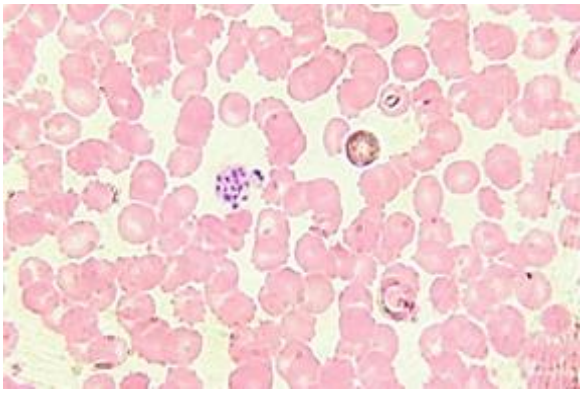

Figure 4: Schizont (plasmodium falciparum)

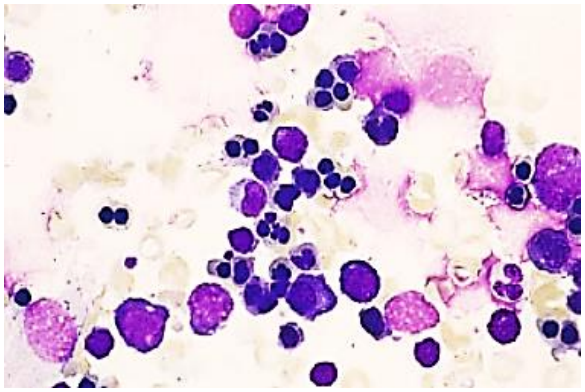

Figure 5: CDA type II- Binucleated erythroblasts

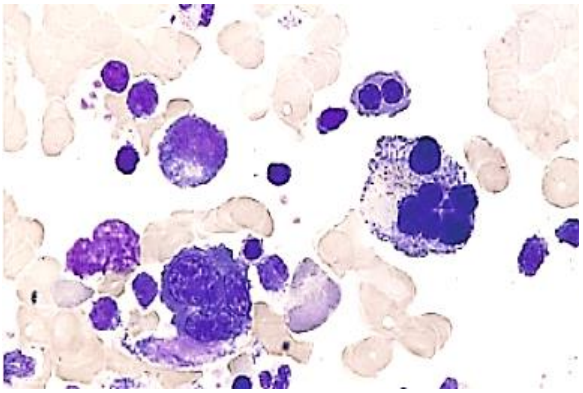

Figure 6: CDA type III – Multinucleated erythroblasts

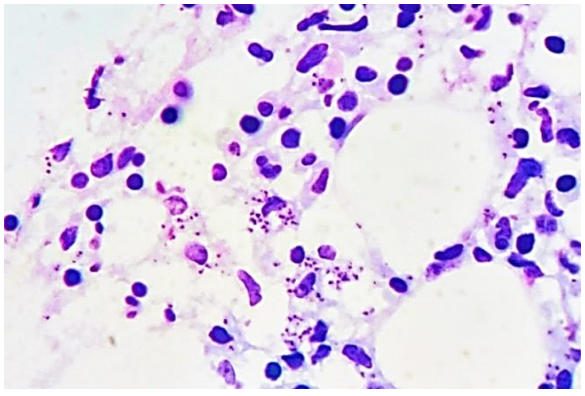

Figure 7: LD body in B.M. biopsy in macrophages

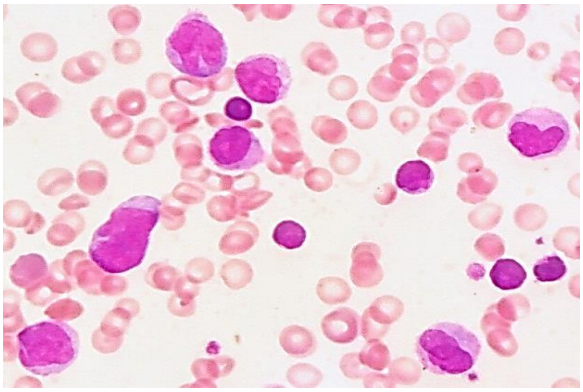

Figure 8: JMML (Monoblasts, Promonocytes, Myeloblast)

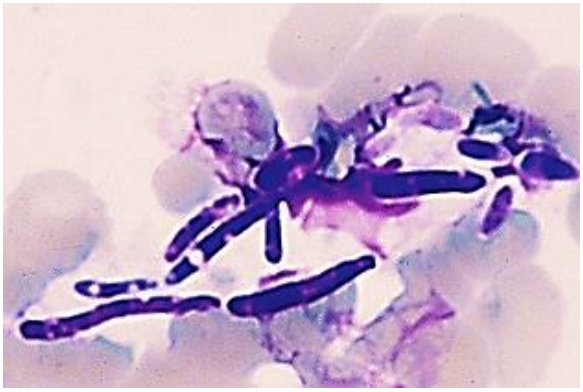

Figure 9: Fungal infection (candida)

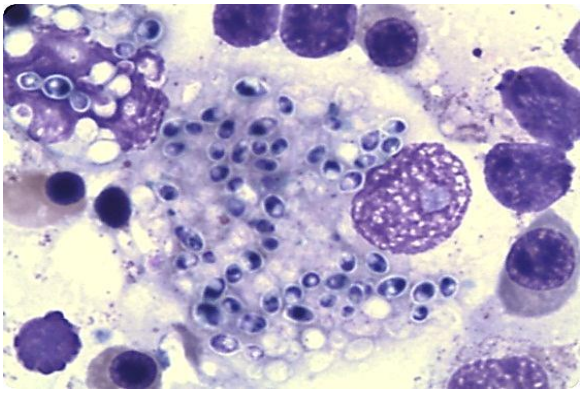

Figure 10: Histoplasmosis in bone marrow

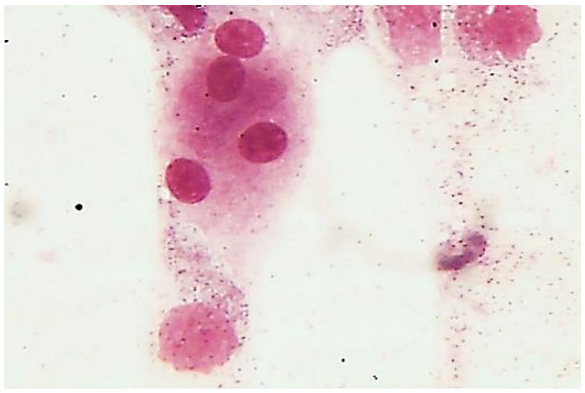

Figure 11: MDS showing pawn ball megakaryocytes

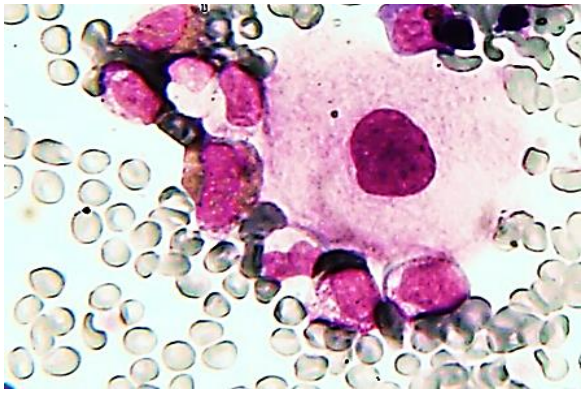

Figure 12: Mononuclear Megakaryocytes

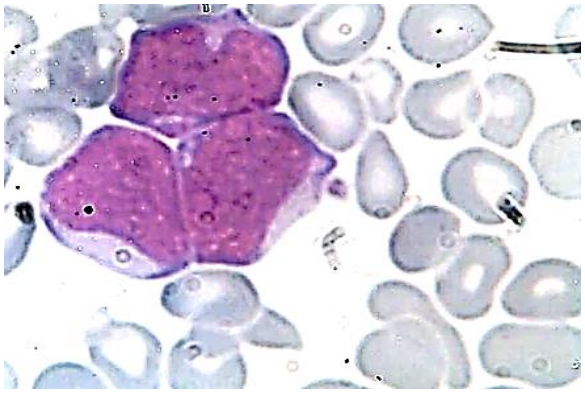

Figure 13: AML – M1

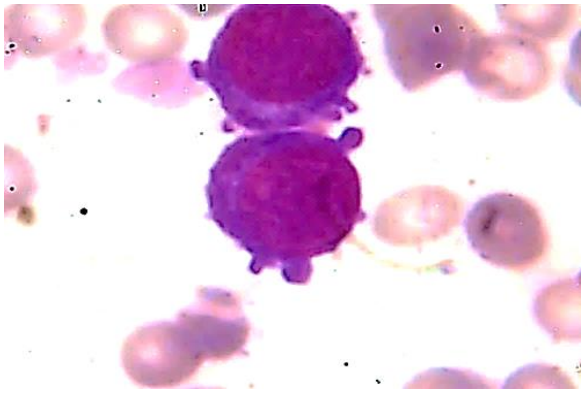

Figure 14: AML – M7

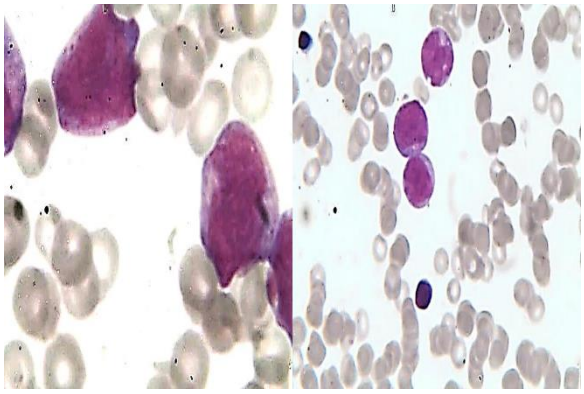

Figure 15: AML-M4 Myeloblast and monocyte

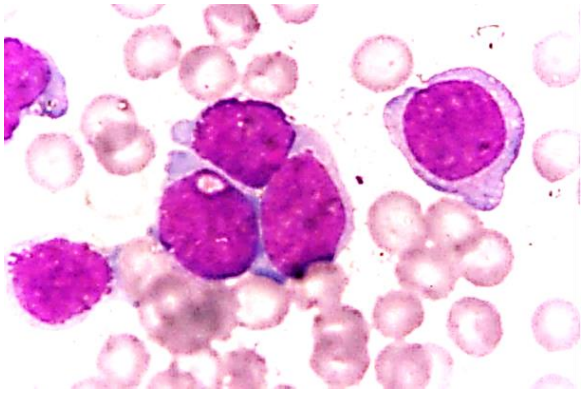

Figure 16: AML –M6 (Trinucleate Erythroblasts)

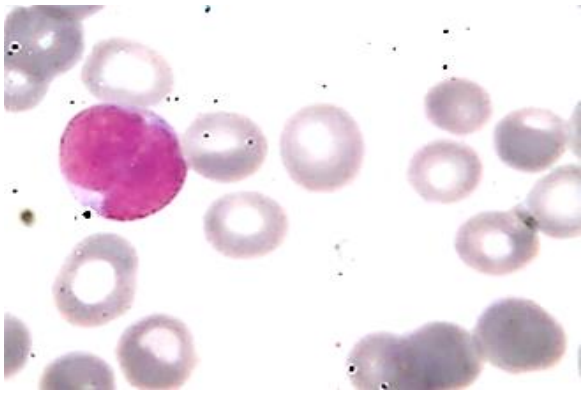

Figure 17: B-ALL (Lymphoblasts with cleaved nuclei)

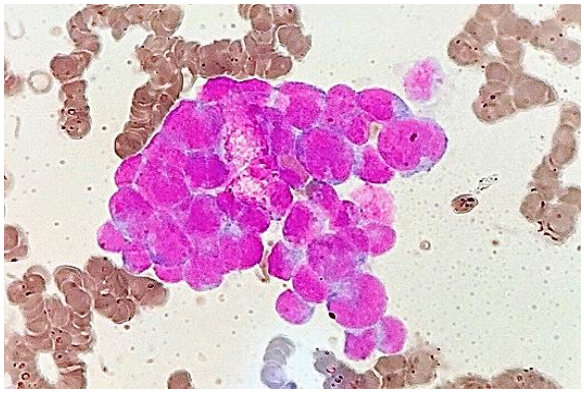

Figure 18: Metastatic Neuroblastoma cells with Rossette formation

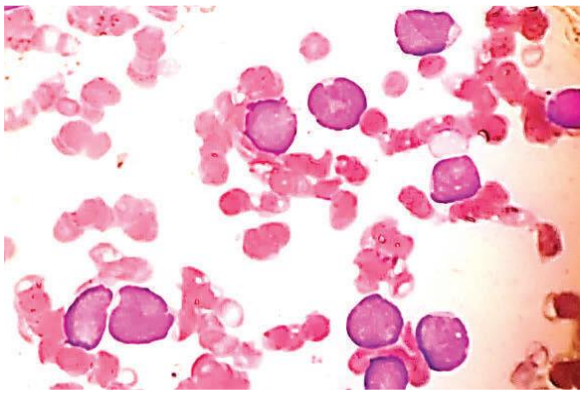

Figure 19: T-ALL

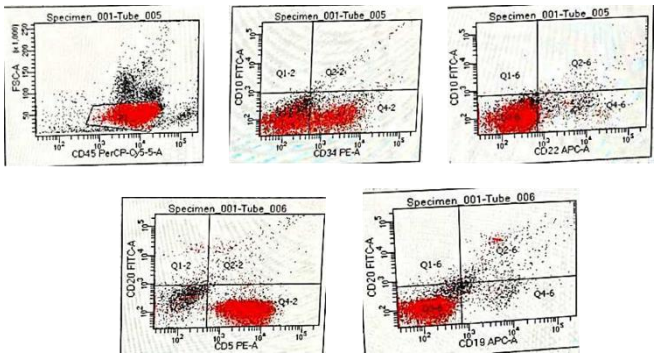

Figure 20: Flow cytometry images 1(A,B,C,D,E):- T-ALL (Strong +ve for CD5, CD34, CD45, -ve for CD19,CD22)

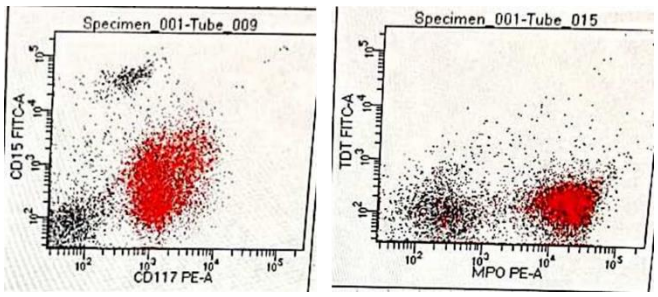

Figure 21: Flow cytometry image 2 (A,B) :- AML (+Ve for CD117, MPO)

Table 4: Comparative Analysis of etiology of pancytopenia in various diseases

Author	No of cases	Most common	2nd Most common
Kumar et al [4] (2001)	166	Aplastic Anemia (29%)	Megaloblastic Anemia (22%)
Nazi et al [10] (2004)	89	Aplastic Anemia (38%)	Megaloblastic Anemia (24%)
Gupta et al [12] (2008)	105	Aplastic Anemia (43%)	Acute Leukemia (25%)
Kungar [5] et al	100	Megaloblastic Anemia (72%)	Aplastic Anemia (14%)
Gayathri et al [11] (2011)	104	Megaloblastic Anemia (74%)	Aplastic Anemia (18%)
Naseem et al [9] (2011)	571	Aplastic Anemia (43%)	Megaloblastic Anemia (13%)
Manzoor et al [8] (2012)	50	Megaloblastic Anemia (56%)	Aplastic Anemia (14%)
Present study (2021)	500	ALL (44%)	Aplastic Anemia (20%)

In the present study most common cause of pancytopenia in bone marrow aspiration study is ALL (44%) & the 2nd common cause was Aplastic anemia (20%).

The high percentage of ALL in this study is due to fact that this study was conducted in pediatric tertiary care referral hospital, so all the cases of leukemia were sent to this hospital for diagnosis, subtyping, and treatment of leukemia cases. So, leukemia forms the majority in this study. It was also noted that pediatric leukemic cases presented with pancytopenia which is different from adult cases which usually presents with leukocytosis. This explains the leukemic predominance & hypercellularity of bone marrow in the present study.

Few rare and interesting cases presenting with pancytopenia is highlighted here.

A case of AML- M6b (Acute erythroid leukemia) in 2 months old child presenting with hepatosplenomegaly, ascites and fever. A provisional clinical diagnosis of hemophagocytic syndrome/ leukemia was made.

1 ½ year old male patient presented with generalized lymphadenopathy, massive, Hepatosplenomegaly. On physical examination child had multiple hypopigmented patches on lower extremities, light colored hair and generalized Lymphadenopathy Peripheral smear shows giant prominent lilac to purple granules in neutrophils, diagnosed as CHS.

Even though Familial and Secondary HPS are rare entities described in literature, but in our study, it constituted 8.3% of total benign cases. 05 cases were Hereditary HPS showing familial preponderance and mutations in PRF1 gene [1,2]

Conclusion

Bone Marrow Aspiration is a reliable and sensitive test which can be used in investigation of Pediatric pancytopenia's and if used meticulously with other routine hematological tests can give a high diagnostic accuracy.

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Conflicts of Interests: None

Reference

1. Dokal IS, Hoffman R. Chapter 12: Inherited aplastic anaemia/bone marrow failure syndromes in Postgraduate Haematology by Victor Hoffbrand, Daneal Catovsky. 2007;6:186-205.
2. Judith CWM, Young NS. Chapter 13: Acquired aplastic anaemia in Postgraduate Haematology by Victor Hoffbrand, Daneal Catovsky. 2007;6:206-225.
3. Agarwal R, Bharat V. Clinical and haematological profile of pancytopenia. International Journal of Clinical Biochemistry and Research. 2015;2(1):48-53.
4. Kumar R, Kalra SP, Kumar H, Anand AC, Madan H. Pancytopenia-A six year study. JAPI 2001;49:1078-81
5. Khunger JM, Arculselvi S, Sharma U, Ranga S, Talib VH. Pancytopenia: a Clinico-haematological study of 200 cases. Indian J Pathol Microbiol. 2002;45(3):375-9.
6. Tilak V, Jain R. Pancytopenia-A clinico-hematological Analysis of 77 cases. Indian J Pathol Microbiol 1999;42:399-404

7. Khodke K, Marwah S, Buxi G, Yadav RB, Chaturvedi NK,. Bone marrow examination in cases of pancytopenia. J Indian Acad Clin Med 2001;2:55-9

8. Tariq M, Khan N, Basri R, Amin S. Aetiology of pancytopenia. Professional Med J Jun 2010;17(2):252-256

9. Naseem S, Varma N. Paediatric patients with bicytopenia/ pancytopenia: review of aetiologies and clinico-haematological profile at a tertiary centre. Indian J Pathol Microbiol. 2011;54:75–80.

10. Memon S, Salma S, Nizamani MA. Etiological spectrum of pancytopenia based on bone marrow examination by children. J Coll Physicians Surg Pak. 2008; 18:163-7.

11. Rathod GB, Alwani M, Patel H, Jain A. Clinico-hematological analysis of Pancytopenia in Pediatric patients of tertiary care hospital. IAIM, 2015; 2(11): 15-19.

12. Gupta V, Tripathi S, Tilak V, Bhatia BD. A study of clinico-haematological profiles of pancytopenia in children. 2008; 38:241-3.

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