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Research Article

Evaluation of bone marrow aspirate in paediatric patients with pancytopenia: a 2 years study

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ABSTRACT

Background: Pancytopenia is an important clinico-hematological entity, characterized by a triad of Anemia, leucopenia and thrombocytopenia. It's not a disease entity itself but a manifestation of many serious and life-threatening diseases. The criteria for defining Pancytopenia is Hemoglobin (HGB) <9 g/dL; total leukocyte count (TLC) <4,000 / µL or absolute neutrophil count (ANC) is < 1.5x 10^9/ L; platelet count, <100 x 10^9/ L. The main objective of this study is to classify pancytopenic cases on etiological basis.
Methods: A total of 750 bone marrow smears of pancytopenic patients were studied.
Results: It comprised of 72 % boys & 28 % girls with the highest number of cases < 5 years of age (64%).The maximum number of cases were of ALL and Aplastic anemias.
Conclusions: CBC, clinical findings and PBS provides valuable information in the workup of pancytopenic patients and help in planning additional investigations on bone marrow samples. Bone marrow evaluation is a valuable diagnostic procedure which may confirm the diagnosis of suspected cytopenias. So the take home message is a) pediatric leukemias unlike adults, usually presents with pancytopenia b) CBC+ PBS + BMA with flow cytometry can help in diagnosing majority of pancytopenias.

Keywords: Aplastic anemia, Megaloblastic anemia, Pancytopenia, Anemia, Leukemia

INTRODUCTION

Pancytopenia is an important clinico-haematological entity, characterized by a triad of Anaemia, leukopenia and thrombocytopenia.[1,2] It's not a disease entity itself but a manifestation of many serious and life-threatening diseases.

The criteria for defining Pancytopenia is Haemoglobin (HGB) <9 g/dL; total leukocyte count (TLC) <4,000 / µL or absolute neutrophil count (ANC) is < 1.5 x 10^9L; platelet count, <100 x 10^9/L.[3] The pancytopenia was labelled as severe if patient had two or more of the following: Hb <7 gm%, ANC <0.5 x 10^9/L, and platelet count < 20 x 10^9/L.[3]

Pancytopenia may result from number of disease processes which affect bone marrow either primarily or secondarily. It can be due to reduction in hematopoietic cell production in the bone marrow by means of infections, malignant cell infiltration or suppression, chemotherapy, radiotherapy and parasitic infestation. The incidence of different disorders causing pancytopenia is variable according to geographical distribution and genetic variation.[4]

Patient with pancytopenia presents with different clinical features. Marrow cellularity and composition in cases of pancytopenia differ in relationship to underlying pathological condition. The marrow is generally hypocellular in cases of pancytopenia caused by a

primary production defect. Cytopenias resulting from ineffective haematopoiesis, increased peripheral utilization or destruction of cells, and bone marrow invasive processes are usually associated with a normocellular or hypercellular marrow.

Differential diagnosis of Pancytopenia

Pancytopenia with hypocellualar bone marrow[5,6]

Inherited aplastic anaemia (Fanconi's anaemia and others) Aleukemic leukaemia's (AML, ALL), MDS Infections.

Pancytopenia with cellular bone marrow

Aleukemic/subleukemic leukaemia's (ALL, AML), Myelofibrosis, Vitamin B12, Folate deficiency, Secondary to systemic diseases (SLE, sjogrens).

The main objective of this study is to evaluate haematological parameters in pancytopenic cases and to classify pancytopenic cases on etiological basis. The present study has been undertaken to evaluate the various causes of pancytopenia and to correlate the peripheral blood findings with bone marrow aspirate.

METHODS

This is a 2 years study (September 2010 to August 2012) carried out in a tertiary care referral paediatrics hospital. Pancytopenia cases were selected based on the criteria.

Inclusion criteria

The criteria for defining Pancytopenia is haemoglobin (HGB) <9 g/dL; total leukocyte count (TLC) <4,000 / µL or absolute neutrophil count (ANC) is < 1.5 x 10^9L; platelet count, <100 x 10^9/L.[3] The pancytopenia was labelled as severe if patient had two or more of the following: Hb <7 gm%, ANC <0.5x10^9/L, and platelet count < 20 x 10^9/L.[3]

Exclusion criteria

(i) Inadequate sample, (ii) inconclusive results. Based on the CBC & PBS findings pancytopenic cases were further reviewed for Bone Marrow Aspiration.

In aplastic anaemia there must be two of the following:[1,2]

1. Haemoglobin below 10 g/dL
2. Platelet count below 50 x 10^9/L
3. Neutrophil count below 1.5x 10^9/L.

Disease severity of aplastic anaemia:[1,2]

1. *Severe aplastic anaemia*:

a) Bone marrow cellularity < 25%, or 25– 50%, < 30% residual haemopoietic cells.

b) Two out of the three following: Neutrophils <0.5 x10^9/L; Platelets <20 x10^9/L; Reticulocytes <20 x 10^9/L.

2. *Very Severe aplastic anaemia*:

   As for severe aplastic anaemia but neutrophils < 0.2 x 10^9/L.

3. Non-Severe aplastic anaemia:

   Patients not fulfilling the criteria for severe or very severe aplastic anaemia.

Statistical analysis of data

All data were expressed as Mean +/ - SD. Statistical analysis was done using unpaired students t test. A level of p value <0.05 was used to indicate statistical significance in all analyses.

Immunophenotyping by flow cytometry

For every diagnosed case of leukaemia immunophenotyping was done by flow cytometry. For problematic cases like MDS, flow cytometry & Molecular genetics were done.

RESULTS

Total number of pancytopenic cases = 750.

Table 1: Sex wise incidence.

Gender	Males	Females
BMA (750)	540(72%)	210(28%)

This study comprises 72% males & 28% females.

Table 2: Age wise incidence.

Age (years)	< 5	5-10	>10
BMA (750)	480 (64%)	250 (33.3%)	20(2.6%)

Majority of paediatric cases belonged to <5 years age group comprising 64% of total cases.

Table 3: Cellularity of bone marrow.

Bone Marrow findings	Total no. of Cases 750
Hypocellular	212 (28.2%)
Normocellular	105 (14%)
Hypercellular	433(57.8%)

The youngest patient in malignant category was a 2 month child diagnosed with AML-M6b & in benign category was 2 months 4 days child with CHS.

In this study pancytopenia with hypercellular B.M. forms the majority comprising 57.8% of total cases. The predominant blood picture was Normocytic normochromic anaemia (41.5%), followed by macrocytic anaemia (33.5%). Leukopenia and thrombocytopenia were seen in all cases.

Table 4: Haematological parameters in cases of pancytopenia.

Parameter	HGB (gm/dl)			WBC x 10^9/L			PLT x 10^9/L		
Range	1.5 -3	3-5	5-9	<0.5	0. 5-2.5	>2.5	50-100	<50	<20
BMA (750)	198	400	152	112	355	293	383	291	76

Table 5: Aetiological categorization of pancytopenia in BMA.

Cellularity	Malignant (540)			Non malignant (210)		
	Acute leukemia		Chronic leukemia	Aplastic anemia		RARE
	ALL	AML		Primary AA	Secondary AA	No. of Cases
Hypocellular 212 (28.2%)	0.2% B-ALL	0.66% M6- 0.2% M2-0.4% M7-0.2%		FA = 2.6% DBS= 0.2% AA=16.6%	Malaria=2% Drugs=2% Infection=11%	CHS=0.4% HPS=1%
Hypercellular 433 (57.7%)	361(48%) B-ALL 197(26%) T-ALL 64(8.5%)	AML = 50 (6.6%) M3- 0.6% M4- 3% M5- 4% M6- 0.2% M7- 0.2%	JMML 0.2% cases			MA = 1% MDS =3.3%
Normocellular 105 (14%)	100 (13.3%)	AML 0.66%				

FA = Fanconi Anemia; AA = Aplastic anemia; CHS = Chediac Higashi syndrome; HPS = Hemophagocytic syndrome; MDS = Myelodysplastic syndrome; JMML = Juvenile myelomonocytic leukemia.

Table 6: Comparative analysis of aetiology of pancytopenia in various studies.

AUTHOR	No. of cases	Most common cause	2nd common cause
Gupta et al (2008)[11]	105	Aplastic Anemia (43%)	Acute Leukemia (25%)
Naseem et al (2011)[10]	571	Aplastic Anemia (43%)	Megaloblastic Anemia (13%)
Manzoor et al (2012)[3]	50	Megaloblastic Anemia (56%)	Aplastic Anemia (14%)
Gayathri et al (2011)[7]	104	Megaloblastic Anemia (74%)	Aplastic Anemia (18%)
Nazi et al (2004)[12]	89	Aplastic Anemia (38%)	Megaloblastic Anemia (24%)
Kungar et al[8]	100	Megaloblastic Anemia (72%)	Aplastic Anemia (14%)
Kumar et al (2001)[3]	166	Aplastic Anemia (29%)	Megaloblastic Anemia (22%)
Present study (2015)	750	ALL (71.73%)	Aplastic Anemia (22.6%)

DISCUSSION

Pancytopenia is an important clinico-haematological entity, characterized by a triad of Anaemia, leukopenia and thrombocytopenia.[1,2] It's not a disease entity itself but a manifestation of many serious and life-threatening diseases.

In this study pancytopenia with hypercellular B.M. forms the majority comprising 57.8% of total cases & Majority of paediatric cases belonged to <5 years age group comprising 64% of total cases. These findings were in correlation with the other studies.[7-9]

The criteria for defining Pancytopenia is haemoglobin (HGB) <9 g/dL; total leukocyte count (TLC) <4,000 / µL or absolute neutrophil count (ANC) is <1.5 x 10^9L; platelet count, <100 x 10^9/L.[3] The pancytopenia was labelled as severe if patient had two or more of the following: Hb <7 gm%, absolute neutrophil count <0.5x10^9/L and platelet count < 20 x 10^9/L.[3] Of the 750 cases studied for BMA, 72% were males & 28 % were females. These findings were in correlation with the other studies.[7-9] The youngest patient in malignant category was a 2 month child diagnosed with AML-M6b & in benign category was 2 months 4 days child with CHS.

The predominant blood picture was Normocytic normochromic anaemia (41.5%), followed by macrocytic anaemia (33.5%). Leukopenia and thrombocytopenia were seen in all cases.

In the present study most common cause of pancytopenia in bone marrow aspiration study is acute leukaemia (71.73%) & the 2nd common cause was aplastic anaemia (22.6%).

The high percentage of ALL in this study is due to fact that this is terminal referral tertiary care hospital for leukaemia. The most striking feature of this study is that 72% of the total cases were leukaemias & ALL is the most common of the entire leukaemia's comprising 61% of the total cases.

This findings is due the fact that this study was conducted in tertiary paediatric and referral hospital so all the cases of leukaemia were sent to this hospital for diagnosis, subtyping and treatment of leukaemia cases. So leukaemia forms the majority in this study. It was also noted that paediatric leukemic cases presented with pancytopenias which is different from adult cases which usually presents with leucocytosis. This explains the leukemic predominance & hypercellularity of bone marrow in the present study.

ALL comprises 61 % of total cases which stands in correlation with other studies.[1-4] Primary Aplastic anaemia comprises (22.6%) of total cases & forms the second most common cause of pancytopenia. There were few cases of secondary AA with known causes like drugs, sickle cell anaemia in aplastic crises.

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

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

WHO (2008) defined AML M6 in B.M. findings

Acute erythro leukaemia (M6a) = Erythroid precursors > 50% and > 20% of NEC as myeloblasts. Pure Erythroid leukaemia (M6b) = > 80 % of Erythroid precursors, Dyserythropoiesis being prominent, Peripheral smear shows Polychromasia, few fragmented RBCs, Basophilic stippling, Few NRBC (5-6/100WBC), Retic Count- 5.2%, WBC- shows 3% blasts + 10% atypical lymphocytes, PLT is reduced, Bone marrow aspirate shows 85% of Erythroblasts with few proerythroblasts. Immunophenotyping by flow cytometry shows about 35% of cells are blasts positive for CD 13, 33, 4, 7, 71, 38, glycophorine, CD 117. AML M6 is a rare entity and its subtype M6B in 2 months old is rarest of rare case.

2. 1 ½ year old male patient presented with generalized lymphadenopathy, massive, Hepatosplenomegaly. On physical examination child had multiple hypopigment patches on lower extremities, light colored hair and generalised Lymphadenopathy Peripheral smear shows giant prominent liliac to purple granules in neutrophils, band forms, lymphocytes and monocytes.

Bone marrow aspiration is hypercellular showing giant prominent grey blue to purple heterogeneous granules often multiple seen in many myeloid precursors, Neutrophils, few lymphocytes and monocytes. Erythropoiesis, myeloid series and megakaryocytes are mildly increased, hemophagocytosis noted.

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