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To study haematological and bone marrow laboratory features presentation in pancytopenia

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Abstract
Background and Objectives: Pancytopenia is a relatively common haematological entity and causes range from simple drug induced bone marrow suppression, megaloblastic haemopoiesis to fatal overwhelming infections and leukaemia. Identification of the correct cause will help in institution of appropriate therapy and prognostication. And to note the spectrum of clinical presentation due to various causes. To identify and document laboratory features in various causes of pancytopenia to analyse the clinico-pathological profile of patients with pancytopenia.
Methods: Total 70 patients aged above 18 years whose haemogram showed pancytopenia were studied.
Exclusion criteria: Patients on chemotherapy, radiation therapy for various malignancies and in whom bone marrow aspiration/ biopsy was not done were excluded.
Results: 46 were males and 24 were females. Youngest patient being 18 years old and the eldest was 73 years old. The common causes for pancytopenia were megaloblastic anaemia 24.8%, aplastic anaemia 17.142%, leukaemia (14.28%) and hypersplenism 11.42%). No definite cause could be identified in 1 case. The common haematological abnormality observed were a macrocytic, hypochromic anaemia with hypersegmented neutrophils on peripheral blood smear and bone marrow showed hypercellularity with megaloblastic erythropoiesis. Bone marrow aspiration was conclusive in most of the cases but in 8.33% bone marrow biopsy was necessary to establish a diagnosis.
Interpretation and conclusion: Common causes of pancytopenia were megaloblastic anaemia, aplastic anaemia, leukaemia and hypersplenism. Severe thrombocytopenia and bleeding manifestation was more commonly associated with aplastic anaemia and leukaemia. Bone marrow biopsy was needed in 8.33% of the cases to establish a diagnosis.

Keywords: Haematological, bone marrow, aspirate/biopsy in pancytopenia

1. 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. Cytopenia is a disorder in which production of one or more blood cell types consensus or greatly reduced [1, 2]. It is not a disease entity, but a triad of findings that may result from a number of disease processes primarily or secondarily involving the bone marrow. The presenting symptoms usually anaemia, leukopenia or thrombocytopenia [3].
Pancytopenia is a striking feature of many serious and life threatening illnesses ranging from simple drug induced bone marrow hypoplasia, megaloblastic marrow to fatal bone marrow aplasia and leukaemia. [4] The pattern of diseases leading to pancytopenia is expected to vary in different population groups with their difference in age pattern, nutritional status and prevalence of infective disorder [4]
Careful assessment of the blood elements is often the first step in assessment of haematologic function and diagnosis of disease. Physical findings and peripheral blood picture provide valuable information in the work up of pancytopenia patients and help in planning investigations on bone marrow samples.[5]Bone marrow evaluation is an invaluable diagnostic procedure in practice of medicine which may confirm the diagnosis of suspected cytopenia, from the clinical features and peripheral blood examination or occasionally give a previously unsuspected diagnosis [6]
The severity of pancytopenia and the underlying pathology determine the management and prognosis of these patients. [5].

In India, the causes of pancytopenia are not well defined'. Previous studies done in India stress the important of megaloblastic anaemia as being major cause of pancytopenia. [5, 7] So the present study has been undertaken to evaluate the various causes of pancytopenia and to correlate the peripheral blood findings with bone marrow aspirate. There by, this data would help in planning the diagnostic and therapeutic approach in patients with pancytopenia.

2. Objectives

1. To study Hematological and peripheral smear presentation of pancytopenia in various clinical cases.
2. To study the special features seen in bone marrow examination.

3. Methodology

The present study was done in MVJ Medical College & Research Hospital, Bangalore. The study period extended between July 2016 to Aug 2018. The study spanned over period of 3 years. Patients admitted to the hospital and whose haemogram showed we pancytopenia were considered for inclusion in the study if they met the inclusion and exclusion criteria. Detailed history and chemical examination of all patients were performed according to proforma.

Diagnostic criteria for pancytopenia: Haemoglobin level < 13.5g dl/males and <11.5g/dl in females; Leukocyte count $<4x10^9$/L; Platelet count $<150x10^9$/L.

Inclusion criteria: Both male and female patients aged more than 13 years presenting features of pancytopenia that is: Hb less than 9 g/dl, TLC less than < 4 x 10^9/L, Platelets count less than 100 x 10^9/L

3.1 Exclusion Criteria: Patients less than 13 years both male and female patients are myelotoxic chemotherapy

Following investigations were done: -

3.2 Complete Haemogram

Three ml of blood was collected by venepuncture under aseptic precaution in a bulb containing ethylene diamine tetra acetic acid (EDTA) anticoagulant. Peripheral smear was stained by leishman stain for all the cases and examined in detail. Haemoglobin estimation (cyanmethaemoglobin method):

Twenty µl of blood was added to 5 ml of drabkin's solution and mixed well. After 10 minutes, the absorbance of the solution was read against a reagent blank at 540nm.. A standard curve was prepared using cyanmethemoglobin graded dilutions of a reference solution of known concentration. Normal value 13 ± 1.5g/dl.

Table 1: Investigations Done In Pancytopenic Patients

Investigations	Method
Bleeding time	Duke's method
Clotting time	Lee and white method
Clot retraction	Tube method
Haemoglobin estimation	Cyanmethemoglobin method
Red blood cell count	Visual counting with Neubauer counting chamber using appropriate diluting fluids
Differential leucocyte count	
Platelet count	
Reticulocyte count	Brilliant cresyl blue
Erythrocyte sedimentation rate	Wintrobe method/ Westergren method
Packed cell volume	Wintrobe method

3.3 Total white cell count

Blood was drawn into the pipette exactly upto the mark 0.5, WBC diluting fluid was then drawn upto the mark 11. Fluid in the bulb was mixed by rapidly rotating the pipette between the fingers. The first few drops from the pipette was discarded. The Neubauer counting chamber was charged. The ruling was brought into focus using the low power objective. The cells in the four large corner squares (4sq.mm) of the Neubauer ruling were counted (n) and WBC count was calculated by the basic formula

= No. of cells counted x dilution factor x depth factor, Area counted = n x 50 cells/ mm^3

Normal value = 4000 – 11000 cells / mm^3

3.4 Platelet Count

Thomas RBC pipette was used. Blood was drawn upto mark 1. Platelet diluting fluid was then drawn upto mark 101. After 2 minutes Neubauer chamber was charged and placed under a petri dish with moist filter paper for 20 minutes. This allows the platelets to settle on the surface of the counting chamber. The moist filter paper keeps the air moist and prevent drying of the chamber. Platelets appear as highly refractile particles under the high power lens.

Platelets were counted in central square, inch of which was divided into 16 small squares. The calculations were done by the formula

= No. of cells counted x dilution factor x depth factor, Area counted = n x 1000 cells / mm^3

Normal value - 150-450 x 10 mm^3

3.5 Bone Marrow Aspiration : Bone marrow aspiration was performed in all the patients using Salah needle after obtaining written consent for the procedure either from the patient or the guardian. The aspiration site was prepared, cleaned with an antiseptic (spirit and betadine), scrubbed and draped, exposing only the aspiration site. Skin and the area down to periosteum was infiltrated with 2 ml of local anaesthetic (2% lignocaine) using 5 ml syringe. The needle with stylet in place was introduced into the site by gentle screwing motion, after adjusting the guard to appropriate length.

The aspirated was transferred to a set of slides and films were prepared by crushing the marrow particles. The needle was withdrawn and the puncture site was sealed with tincture benzoin swab. In cases of unsuccessful attempts of bone marrow aspiration, a repeat aspirate was done at the

different site and only cases where diagnostic aspirate could be obtained have been included in the study.

Slides were fixed in methanol for 15 minutes, dried and later stained with Leishman stain and marrow aspiration smears were examined for Cellularity, M: E ratio, Erythropoiesis, Myelopoiesis, Megakaryopoiesis, Other-plasma cells, lymphocytes, mast cells, Parasites, Abnormal cells. Special stains were done whenever required. Biopsy can be done at same site using same needle or new biopsy needle. Care should be taken to reposition the needle biopsy site at a new place.

4. Results

Hemoglobin percentage varied from 1.8 to 9 gms%. Most patients had hemoglobin percentage of 1.8 to 5gm% lowest was 2.1 in leukaemia.

Total leucocyte count ranged from 500 to 3900 most of the patients had the white cell count ranged from 1001 to 2500. Lowest count was 850 seen in NHL. Platelet count ranged from less than 1000 to 95000. Lowest count was seen in 11,000 in aplastic anemia.

Of the 70 cases of pancytopenia, megaloblastic anemia comprises largest aetiological group that was 17 (24.28%) followed by aplastic anemia 12 (17.42%) and leukaemia 10 (14.28%) respectively. And the aetiolgy could not be detected in 1 case.

Data analysis showed the following results:

Table 2: Vital hematological parameters in pancytopenia

Sl. No.	Parameter	Range	No. Of cases	Percentage (%)
1	Haemoglobin (gm%)	1.8-5	36	51.42
		5.1-8	18	25.72
		8.1-9.0	16	22.86
	Total		70	100
2	Total leucocyte (cells/ mm^3)	500 - 1000	7	10
		1001 - 2500	44	62.85
		2501 - 3900	19	27.150
	Total		70	100
3	Platelet count (cells/ mm^3)	Less than 1000	2	0.28
		12000 - 50000	26	37.14
		51000 - 80000	18	25.71
		81000 - 950000	24	34.28
	Total		70	100

Table 3: percentage of pancytopenia cases in various age groups

Age group (years)	No. Of cases	Percentage (%)
16-20	13	18.57
21-30	9	12.85
31-40	19	27.142
41-50	14	20
51-60	7	10
61-70	6	8.57
Above 70	2	2.85
Total	70	100

4.1 Age distribution: In the present study patients of all age groups were noted to have pancytopenia, In the present study most of the cases were in 3rd & 4th decade that is 19 (27.142%) where as only one patient reported with pancytopenia above 70 years mean age of pancytopenia is 38.7 ± 15.1 and the age ranged from 16.73 in the study.

4.2 Sex distribution: In present study the distribution of pancytopenia was found in more in males than females. That is 46/70 (65.71%) in males and 24/70 (34.28%) in females. Mean age for male is 41.7 ± 15.7, mean age for female is 32.5 ± 11.4 and M : F is 2.2 : 1

Table 4: Etiology of pancytopenia in the present study

Etiology	No. of cases	Percentage (%)
Megaloblastic anemia	17	24.28
Aplastic anemia	12	17.142
Leukemia	10	14.28
Hypersplenism	8	11.428
Non-hodgkin's lymphoma	5	7.142
Disseminated TB	4	5.71
HIV	3	4.285
Overwhelming infection (other than TB)	3	4.285
MDS	2	2.857
PNH	3	4.285
SLE	1	1.7
Multiple myeloma	1	1.7
No cause	1	1.7
Total	70	100

Table 5: Peripheral blood picture in pancytopenia patients

Sl no.	Blood picture	No. of cases	Percentage (%)
1.	Atypical lymphocytes	2	3.3
2.	Blast cells	10	14.28
3.	Dimorphic	2	3.3
4.	Macrocytic hypochromic	20	28.57
5.	Macrocytic normochromic	6	8.57
6.	Normochromic hypocellular	13	18.57
7.	Normocytic normochromic	17	24.28

Peripheral blood picture showed macrocytic hypochromic picture showing 20 cases with 28.5% followed by normocytic normochromic picture 13 with 18.57% and least is dimorphic with 2 with 3.3%.

In the present study 19 of 70 cases had megaloblastic anemia. The highest evidence of age is between 3rd and 4th decade. The mean age at presentation of anemia in megaloblastic anemia was 36.3 years. The duration between the onset of first symptom and diagnosis, varied a day to 1-2 years. Average duration of symptoms before detection was 3 months in 5 cases onset was quite in 10 days during two year study.

Incidence of megaloblastic anemia shows strong predilection for male that is 12 out of 19 cases (63.15%) and that of females comprising small group of 7 out of 19 cases (36.84%) ratio with male and female is 2.2:1.

Hemoglobin percentage showed variation from 1.8 to 9.0 gm%. Most of the patients had haemoglobin percentage between 5.1-8gm%, were 10 (52.63%). Total leucocyte count: white blood cell count ranges from 500 – 3,900 cells/mm^3. Most of the cases varied from 1,000 to 2,500 that were 13 (68.42%), of the patients had platelet counts between 81,000 – 95,000 cells/mm^3 that were 10 (52.63%).

Table 6: Peripheral blood picture in Megaloblastic anaemia

Sl. No.	Blood picture	No. of cases	Percentage
1	Macrocytic hypochromic	12	63.15
2	Macrocytic normochromic	5	26.31
3	Normochromic hypochromic	1	6.3
4	Normocytic normochromic	1	6.3
	Total	19	100

Majority of the cases showed macrocytic hypochromic anemia 63.15%, followed by macrocytic normochromic picture which is 26.31%. Neutropenia & thrombocytopenia was noted in all cases. In present study out of 70 cases 13 patients had aplastic anemia, 13 cases of pancytopenia were due to aplastic anemia, That is 23.07% mean age of 38.4 years. Among them 7/11 cases were males and 4/11 cases were females with M: F. ratio of 1.8:1.

Table 7: Symptomatology of patients with aplastic anemia

Symptoms	No. of cases	Percentage
Easy fatigability	3	27.3
Breathlessness	3	27.3
Palpitation	6	54.5
Bleeding manifestation	5	45.5
Fever	4	36.4

The etiology of aplastic anemia remained idiopathic in 10 cases, with 1 case associated with exposure to drugs. The duration of the onset of symptoms to diagnosis was as long as 1½ year and as short as 3 weeks. The common complaint was bleeding only 4/11 presented with fever. All had pallor on presentation and most common feature was bleeding in 5/11 cases.

Table 8: causes of Hypercellular Bone Marrow

Sl. No.	Aetiology	No. of cases	Percentage
1	Hypersplenism	6	37.5
2	Overwhelming infection	3	18.75
3	Aplastic anemia	1	6.25
4	Disseminated TB	1	6.25
5	MDS	1	6.25
6	NHL	1	6.25
7	SLE	1	6.25
8	HIV	1	6.25
9	No Cause	1	6.25

Hypersplenism with pancytopenia: 7 out of 70 cases of pancytopenia were attributed to Hypersplenism. Patients with hypersplenism presented with varied complaints of pain abdomen, abdominal distension and upper/lower GI bleed. The most common curse of hypersplenism with pancytopenia was idiopathic portal hypertension. Next common cause was HbsAg +ive cirrhosis. In the present study of pancytopenia leaving behind megaloblastic anemia, Hypersplenism constitutes 6 cases (37.5%) followed by overwhelming infection that is 3 cases (18.75%) which showed pancytopenia with hypercellular bone marrow.

Table 9: Vital Hematological Parameters in Bone Marrow Hypoplasia

Sl. No.	Parameter	Range	No. of cases	Percentage (%)
1	Haemoglobin (gm%)	1.8-5	11	84.61
		5.1-8	2	18.2
		8.1-9.0	-	-
	Total		13	100
2	Total leucocyte (cells/ mm^3)	500 - 1000	-	-
		1001 - 2500	10	76.92
		2501 - 3900	3	23.07
	Total		13	100
3	Platelet count (cells/ mm^3)	Less than 1000	2	11.76
		12000 - 50000	8	61.53
		51000 - 80000	3	27.3
	Total		13	100

In the present study highest age incidence of hypocellular marrow between 41 - 50 years. Its occurrence was least between age groups 21 – 30 & 61 - 70 and no cases reported in other age groups.

Haemoglobin percentage varied from 1.8 - 9.0 gm%. Most of the patients had 1.8 - 5gm%. Total leucocyte count range was from 500 – 3900, most patients were seen in 1001 to 2500 range. Platelet count ranged from less than 1000 to 80000 and most of the patients ranged from 12000 to 50000.

5. Discussion

Table 10: Physical findings compared to the other studies

	Physical findings								
	Splenomegaly			Hepatomegaly			Lymphadenopathy		
Diseases	A	B	C	A	B	C	A	B	C
Megaloblastic anemia	40	22	2	42	23	1	1	3	1
Aplastic anaemia	--	4	1	1	3	1	0	1	1
Subleukemic leukemia	8	1	3	10	1	3	6	--	1
MDS	4	--	0	4	--	0	0	--	0
Hypersplenism	6	--	5	4	--	1	0	--	0
Malaria	2	2	2	--	--	0	0	--	0
Multiple myeloma	1	1	0	--	--	1	0	--	0
Disseminated tuberculosis	1	1	1	--	1	1	1	1	1
Storage disease	--	--	0	--	--	0	0	--	0
CLL - SLL	2	1	0	2	1	0	2	1	0

A - Khunger J *et al*., B - Tilak *et al*., C - Present Study The variations in frequency of various diagnostic entities causing pancytopenia has been attributed to difference in methodology and stringency of diagnostic criteria, geographic area. period of observation, genetic difference and varying exposure to myelotoxic agents etc. The commonest cause of pancytopenia reported from various studies throughout the world has been aplastic anemia. This is in sharp contrast to our study where commonest cause is Megaloblastic anemia. Similar findings were observed in other studies in India.

This seems to reflect higher prevalence of nutritional anemia in our population.

The most common physical finding was pallor followed by splenomegaly that is 15 (25%), followed by splenomegaly and hepatomegaly with 13 (21.7%) & 13(21.7%) respectively. The age of patients ranged from 16-73 years with mean age of 38.7. Pancytopenia is an investigation finding than a diagnosis. It is accompanying various disease entities. There are very few, study frequencies in India, and also from other parts of the world. Various infections that are localised to geographical area can also cause pancytopenia. In the present study 70 cases of pancytopenia were studied the data analysed were compared with other standard studies. In the present study symptoms due to anemia was noted in all most all cases that is pallor 100%, Easy fatiguability in 68.33% of cases, Fever in 45% of cases, Palpitation in 31.7% of cases, Breathlessness manifested in 35% of cases.

Bleeding as a unique manifestation in the later stages seen in most of the cases that is 45%. The presenting symptoms are usually attributable to thrombocytopenia. Leukopenia was an uncommon cause of the initial presentation of the patient. Bleeding manifestation was common in leukaemia / lymphoma and aplastic anemia. Most of the cases of leukaemia had bleeding as a major manifestation at presentation & other cases, at the later stages of the disease. In the present study, pallor was present in all 100%, the second most common finding was splenomegaly, and megaloblastic anemia, as the leading cause of pancytopenia.

In the present study megaloblastic anemia was noted 16 out of 60 cases, Peripheral smear in megaloblastic anemia showed macrocytes and hypersegmented neutrophils MCV was elevated in most of these patients. Haemic murmur was noted in 7/60 cases and hepatomegaly in 13/60 cases. Bleeding manifestation was noted in 27/60 patients and lymphadenopathy in 13/60 cases. Megaloblastic anemia was the most common cause of pancytopenia in the present study. Similar results have been reported in haematological study from other Indian centers. Nutritional factors, recurrent infection and deficiencies of vit B_{12} and folate seem to be strongly associated with megaloblastic anemia. The mean age in the present study was 38.7 years.

Aplastic Anemia: The mean age of patients in this group was 38.4 years. Male to female ratio was 1.8:1. The etiology of aplastic anemia was idiopathic in 90% of cases. One patient was on native drug which might have induced aplastic anemia. idiopathic forming majority of cases of aplastic anemia, The increased, incidence may be related to environmental factors, such as increased exposure to toxic chemicals rather than genetic factors.

Leukaemia: 9 Cases of leukaemia also presented with pancytopenia. All were diagnosed as having AML. Patients had bleeding manifestations at presentation. Peripheral smear showed blasts and other features consistent with AML.

Hypersplenism: The mean age in the hypersplenism group was 33 year. Male to female ratio was 1.4:1. The splenic size noted in patients with hypersplenism varied from 12cm to 22cm. The mean spleen size was 17cm. Alcoholic cirrhosis commonly presented with pancytopenia due to hypersplenism.

HIV can cause pancytopenia by various ways bone marrow infiltration by opportunistic infection or malignancy vit B or folic acid deficiency, bone marrow suppression by HAART or drugs given for prophylaxis or HIV disease itself can cause pancytopenia. 4 patients with advanced HIV disease presented with pancytopenia. Bone marrow showed finding suggestive of HIV induced suppression in HIV patients it is very important to find the cause of pancytopenia before planning to treat that.

Pancytopenia was noted 2 patients with PNH. Usually have a normocellular bone marrow but patients with PNH may have the aplastic period lasting for weeks to year. Singh *et al* have reported two cases of PNH; both were passing high coloured urine.

In SLE, though haematological abnormalities are frequently encountered haematological presentation per se without other manifestations are rare in SLE.

Bone marrow aspiration showed megaloblastic erythroid hyperplasia. Megaloblasts had the characteristic feature of sieved nuclear chromatin, asynchronous nuclear maturation,

bluish cytoplasm with cytoplasmic blebs. Giant metamyelocytes and band forms were predominant in leukocytic series. 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 haematological assays are 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 in India, the exact deficiency is usually not identified.

6. Summary

- A total of 60-patients whose haemogram showed pancytopenia were studied of which 41 patients (68.3%) were males and 19 patients (31.7%) were females. Youngest patient being 16 years old and the eldest was 73 years. Patients of all age groups were noted to have pancytopenia, but more number of cases was in the 3 and 4th decade.
- The commonest etiological cause noted to cause pancytopenia was megaloblastic anemia (26.67). The next common causes were aplastic anemia (18.30%), leukemia (15%). hypersplenism (11.7%), Other diseases associated were HIV (6.7%), disseminated TB (3.3%), overwhelming infection (3.3%) MDS (1.7%) PNH (3.3%) and SLE (1.7%). No definite cause could be identified in 1.7% of the patients.
- Easy fatiguability was the commonest presenting complaint (68.33%) followed by generalized weakness, bleeding manifestation (45%) and fever (45%). The duration from the onset of symptoms to diagnosis was short as 5 days to as long as two and half years. Out of 60 patients, 46% of patients had involvement of either liver or spleen or both. Pallor was present in all the cases (100%). 50% of patients with leukemia had bleeding manifestation at presentation.
- The hematological parameters such as hemoglobin varied from 1.8 gm% to 9 gm%, TLC varied form 500/cu.mm to 3900/cumm of blood and PLT count varied from <1000/cumm to 95000/cumm. A low mean PLT count was noted in leukaemia.
- A blood peripheral smear showed macrocytic hypochromic anemia with hypersegmented neutrophils as the commonest finding. A bone marrow aspiration showed hypercellularity with megaloblastic erythropoiesis as the commonest finding (26.67%). Only AML was noted to be associated with pancytopenia of all the cases of leukemia diagnosed by bone marrow aspiration/biopsy.
- A bone marrow aspiration was conclusive in most of the patients but in 8.3% of the patients bone marrow biopsy was necessary to establish the etiology of pancytopenia.

7. Conclusion

Pancytopenia is not an uncommon haematological problem encountered in clinical practice and should be suspected on clinical grounds when a patient presents with unexplained anemia, prolonged fever and tendency to bleed.

The physical findings and peripheral blood picture provides valuable information in the work of cytopenic patients.

Evaluation of peripheral blood film reveals the most probable cause of anemia, presence of nucleate RBCs and/or immature myeloid cells may suggest marrow infiltration or primacy hematologic disorder.

Bone marrow aspiration is an important diagnostic tool in hematology which helps to evaluate various cases of cytopenia. Bone marrow examination is an accurate, reproducible, rapidly available information at an economical cost and with minimal discomfort to the patient. Bone marrow aspiration is sufficient to make a diagnosis in cases of nutritional anemias and initial diagnosis of leukemia.

Megaloblastic anemia was the commonest cause which indicates the high prevalence of nutritional anemia in our region. The other common causes were hypoplastic aplastic marrow. However, uncommon and rare causes such as multiple myeloma, storage disease should be kept in mind while planning investigation for complete work up of cytopenic patients. In patients presenting with cytopenia and hepatosplenomegaly, smear revealed but marrow showed megaloblastic change indicating acute vitamin B12 and folate deficiency in patients with malaria infestation

Tuberculosis being highly prevalent and endemic in india, it is essential to be aware off its manifestation as pancytopenia. Present study concludes that detailed primary haematological investigations along with bone marrow aspiration in cytopenic patients is helpful for understanding of the disease process, to diagnose or to rule out the causes of cytopenia and helpful in planning further investigations and management of cytopenic patients.

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