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PATTERN OF DISEASES GENERATED BY FIVE PART CELL COUNTER SCATTERGRAMS IN CASES OF PANCYTOPENIA.

Haematology

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ABSTRACT

Aim- This study observes the patterns generated by Five part cell counter scattergram in various diseases presenting as pancytopenia. **Method-** This is an observational cross sectional study conducted in department of pathology. EDTA blood sample of all newly detected Pancytopenia cases were run in ADVIA 2120i 5-part cell counter and scattergram were obtained. Peripheral smear and bone marrow examination was done as and when needed.Characteristic patterns were observed. **Results-** 120 cases of pancytopenia were included in the study. Majority 27 (22.50%) cases belonged to the age group of 27-36 and a male preponderance was observed. Nutritional anemia was the commonest etiological cause of pancytopenia in our study where megaloblastic anemia was (46.67%) followed by 21.67% cases of dimorphic anemia. Majority of the cases showed macrocytic hypochromic changes in RBC scattergram. RBC scattergram pattern were 95.38% concordant with peripheral smear findings. Numerical indices showed discordance with RBC scattergram findings in cases where MCV was in range of 75-95fL. 93.33% cases showed changes in the normal WBC scattergram pattern with significant p value. **Conclusion-** 5 part hematology cell counter scattergram provide necessary diagnostic evidences in the form of characteristic patterns even before peripheral smear examination. They help in segregating the causes of pancytopenia which require basic treatment from the ones which require extensive tertiary investigative procedures for its mere diagnosis.

KEYWORDS

Pancytopenia, 5 part hematology analyzer, scattergram

INTRODUCTION

Pancytopenia is not a disease by itself but a set of findings which results due to reduction in the three cell components of the peripheral blood .[1] This low concentration of cells causes anemia, leucopoenia and thrombocytopenia where haemoglobin is less than 9 gm%, WBC count of lower than 4000 per mm^3 and platelet count lower than 1.5 lakhs/mm^3 respectively are taken into consideration. [2],[3]

Pancytopenia can occur in various conditions like deficiency of nutrition, haematological neoplastic condition, reduction in bone marrow activity and plenty others.[4] The severity of pancytopenia and its underlying pathological change helps to direct the management and the prognosis of the underlying disease.[5]

Peripheral blood film provide relevant information regarding aetiology. However in severe cases it is very difficult or impossible to make the differential count[4]. With the availability of automated measurement of many cellular indices, numerical parameters and scattergram obtained from hematology cell counter, results can be obtained in minimum span of time and with higher degree of accuracy and precision. Subsequently manual labour demand has also been tremendously reduced.[6],[7]

On scattergrams generated by automated hematology, the cells are plotted according to the size of the cell, the granularity and shape and size of the nucleus. These scattergram are efficient in detecting the possibility of abnormal cell populations and are helpful in providing statuary flags. They are useful in screening and detecting haematological abnormalities in advanced hospitals and health care clinics.[8]

This study is thus undertaken to observe the patterns generated by Five part cell counter scattergram in various diseases presenting as pancytopenia. These scatergram patterns enhance the diagnostic utility of automated data and give clues for diagnosis even before peripheral smear examination.

Aims and Objectives

To study the pattern of diseases generated by Five Part Cell Counter scattergram and evaluate its role in cases of pancytopenia.

MATERIALS AND METHOD

A 2 years observational cross sectional study was conducted in department of Pathology, tertiary health care institute. A total of 120 cases of both sex and all ages with complete blood count (CBC) run on Siemens Advia 2120 five part cell counter having haematological findings of pancytopenia were included in the study.

All newly detected Pancytopenia cases (hemoglobin percentage less than 9gm%, WBC count of lower than 4000 per cubic millimetre, platelet count less than 1.5lakh/mm^3)giving consent for the study were included after Institutional Ethics Committee approval. Pattern of scattergram of these cases were analysed. Complete history, important clinical details, systemic examination findings were noted. Peripheral blood smears and bone marrow aspirations stained with Leishman's stain or Hematoxylin and Eosin stain were studied as an when needed.

RESULTS

- Out of the 120 cases, 69(57.50%) cases were males.
- Average age of the patients ranged from 7-70 years with mean of 37.88 years. Majority of the individuals 27(22.50%) belonged to the group of 27-36 years followed by 25(20.83%) cases in age group of 37-46 years followed by 23(9.17%) cases belonging to the age group of 17-26 yrs. There were 18 cases above 57yrs and only one case >66 years of age.
- The dietary history of the patients showed that 88(73.33%) cases were non-vegetarian and rest 32(26.67%) were vegetarian.

Table 1. Shows Distribution Of Study Subjects According To Mean Hematological Parameters

Hematological parameters	Mean± SD
WBC	2.47±0.93
RBC	4.25±21.07
Hemoglobin	10.49±42.45
Platelet count	47.70±30.16
MCV	93.37±14.06

Table No2. Distribution Of Study Subjects According To Peripheral Smear Findings

PS findings	Number	P ercentage
Dimorphic hypochromic	25	20.83
Dimorphic hypochromic, blasts +	02	1.67
Macrocytic hypochromic	65	54.17
Microcytic hypochromic	18	15.00
Normocytic hypochromic	07	5.83
Normocytic normochromic	03	2.50
Total	120	100

Table No 3. Shows Distribution Of Study Subjects According To RBC Cytogram Findings

RBC cytogram findings	Number	Percentage
Macrocytic hypochromic	62	51.67
Dimorphic hypochromic	32	26.67
Microcytic hypochromic	17	14.17

Normocytic hypochromic	09	7.50
Total	120	100

Table No.4 Concordance Between Peripheral Smear And RBC Cytogram Find

RBC cytogram findings	Peripheral smear findings				
	Macro-cytic hypo-chromic	Dimor-phic hypo-chromic	Micro-cytic hypo-chromic	Normo-cytic normo-chromic	Total
Macrocytic hypochromic	62	00	00	00	62
Dimorphic hypochromic	03	25	02	00	32
Microcytic hypochromic	00	00	16	00	17
Normocytic hypochromic	00	00	00	07	09
Total	65	25	18	07	120
Concordance	95.38%	100%	88.89%	100%	

Table 5 . Distribution Of Study Subjects According To Cellularity On Bone Marrow

Cellularity on bone marrow	Number	Percentage
Hypercellular	81	67.50
Hypocellular	08	6.67
Normocellular	11	9.17
Bone marrow not done	20	16.67
Total	120	100

Table No 6. Distribution Of Study Subjects According To Final Diagnosis

Final diagnosis	Number	Percentage
Iron deficiency anemia post transfusion	01	0.83%
Aplastic anemia	07	5.83%
Dimorphic anemia(s/o combined nutritional deficiency)	26	21.67%
Iron deficiency anemia	07	5.83%
Iron deficiency anemia with ITP	01	0.83%
Megaloblastic anemia	56	46.67%
Megaloblastic anemia with ITP	01	0.83%
Multiple myeloma	01	0.83%
Myelodysplastic syndrome (MDS)	06	5.00%
Reactive to infection (bacterial)	02	1.67%
Reactive to infection (dengue)	09	7.50%
Subleukemic leukemia (ALL)	01	0.83%
Subleukemic leukemia (ALL-L1)	01	0.83%
Subleukemic leukemia (AML- M3)	01	0.83%
Total	120	100.00%

Out Of The 120 Cases, Only 08(6.67%) Cases Showed A Normal WBC Scattergram Whereas 93% Cases Showed Scattergram Changes.

Table No. 7 Distribution Of Study Subjects According To Abnormality On Scattergram

Abnormality on scattergram	Number	Percentage
Shift to left in neutrophil area	62	51.67
Widening of RBC ghost area	38	31.67
Widening of Eosinophil area	30	25.21
Rise in LUC area	10	8.33
Dense blast area	05	4.17
Widening of neutrophil area	02	1.67
Total	120	100

Table No.8 Association Between Abnormal Scattergram Findings And Megaloblastic Anemia

Abnormal scattergram findings	n	Final diagnosis		χ^2 p value
		Megaloblastic anemia	Other diagnosis	
Shift to left in neutrophil area	62	42	20	0.00004*
Widening of RBC ghost area	38	23	15	0.05
Widening of Eosinophil area	30	19	11	0.05

Table No. 9 Association Between Abnormal Scattergram Findings And Cases Reactive To Infection

Abnormal scattergram findings	n	Final diagnosis			χ^2 p value
		Cases reactive to infection (bacterial)	Dengue	Other diagnosis	
Widening of neutrophil area	02	02	00	00	0.0000*
Rise in LUC area	10	00	07	03	0.0000*

Table No. 10 Association Between Abnormal Scattergram Findings And Sub Leukemic Leukemia

Abnormal scattergram findings	n	Final diagnosis		χ^2 p value
		Sub leukemic leukemia	Other diagnosis	
Rise in LUC area	10	03	07	0.00001*
Dense blast area	05	03	02	0.00001*

Table No. 11 Association Between Abnormal Scattergram Findings And MDS

Abnormal scattergram findings	n	Final diagnosis		χ2 p value
		MDS	Other diagnosis	
Dense blast area	05	02	03	0.01*

DISCUSSION

Pancytopenia is not a disease per se but a combination of findings as a result of various disease processes. The severity of the pancytopenic condition and its underlying pathological etiology helps to direct the management and the prognosis of the underlying disease. Automated hematology analysers provide scattergram which show characteristic patterns and give clues about the etiology of pancytopenia especially in case of severely pancytopenic patients when the cell counts are reduced.[4] The present study included 120 cases of pancytopenia. We observed the pattern of diseases presenting as pancytopenia in a Five part cell counter scattergram.

Age Incidence and Gender

Analysis of demographic profile of our study shows that mean age of the patients was 37.88± 15.14 with range of 7-70 years. Majority 27(22.50%) cases belonged to age group of 27 to 36 years followed by 25(20.83%) cases of age 37-46 years. Majority 69(57.50%) cases were males and 51(42.50%) were females. These findings were broadly in line with other studies by Makheja et al[9], Azaad et al[10], Dasgupta et al[11], Pereira et al[5], Mansuri et al[12], Deepankar et al[13], Chandra et al[14], Anjum et al[15], Chohan et al[16] and Sandhya et al [17].

Type of Diet

In present study, out of 120 cases, 88(73.33%) cases were non-vegetarian and rest 32(26.67%) were vegetarian. Deepankar et al[13] in their study on megaloblastic anemia found maximum 63% cases as non-vegetarian while 37% as vegetarians. This is comparable to present study findings in which megaloblastic anemia was found in 67% non-vegetarians and 33% vegetarians. Although various studies show association of megaloblastic anemia and Vitamin B12 deficiency with vegan diet, a study conducted by Shrivastava S et al[18] concluded that use of non-vegetarian food from processed sources in the form of fast food outlets and changes in the conservative cooking habits have increased the risk of developing megaloblastic anemia in non-vegetarians as well.

Final Diagnosis/ Cause of Pancytopenia:

In present study, maximum 56(46.67%) cases had megaloblastic anemia followed by 26(21.67%) cases of dimorphic anemia which were suggestive of combined Vit B12/folate and iron deficiency. This was followed by cases reactive to infection where there were 09(7.50%) cases of dengue followed by iron deficiency anemia in 07(5.83%) cases. The studies of Gayathri et al[19], Azaad et al[10], Mansuri et al[12], Chohan et al[16], Pereira et al[5] and Modi et al[4] also showed megaloblastic anemia as the commonest etiology of pancytopenia.

Peripheral Smear Findings

65 out of 120 cases(54.17%) cases had macrocytic hypochromic anemia followed by 25(20.83%) cases with dimorphic hypochromic anemia. There were 18(25%) cases having microcytic hypochromic anemia.

Bone Marrow Findings

In the present study, we found maximum 52 cases (43.33%) showing

megaloblastic erythropoiesis on bone marrow aspiration followed by 24(20%) cases with both megaloblastic and micro-normoblastic erythropoiesis. The findings of the present study was highly similar to the findings of studies by Gayathri et al[19], Makheja et al[9], Azaad et al[10], Dasgupta et al[11], Modi et al[4], Anjum et al[15]. In 20 cases (16.67%), bone marrow aspiration could not be done.

RBC Cytogram Findings

In present study we observed that majority 62(51.67%) cases had macrocytic hypochromic anemia followed by 32(26.67%) cases with dimorphic hypochromic anemia. There were 17(14.17%) cases having microcytic hypochromic anemia. These findings were comparable with the study of Sandhya V et al[17] where the most common finding like the present study was macrocytic hypochromia however their second most common finding was normocytic normchromic as the second most common cases were of aplastic anemia representing a normocytic normochromic population.

A study conducted by Kakkar N et al[21] distributed the cases according to the volume of red blood cell and concentration of hemoglobin in rbc scattergram/cytogram. The difference in size and hemoglobin content leads to different scatter patterns which are characteristic for diseases presenting as pancytopenia. In megaloblastic anemia, majority of red blood cells have a higher volume and they show significant variation in shapes of RBCs in the form of macro-ovalocytes. The RBC cytogram in patients of megabloblastic anemia shows cell plots in the zone of macrocytic cells (Fig 1).

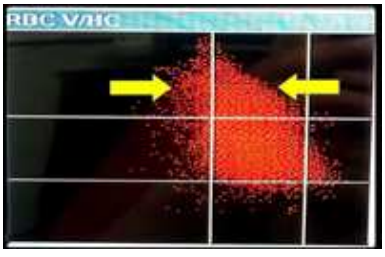

Fig No 1. - RBC Scattergram In Megalobalstic Anemia.

In cases of non-megaloblastic macrocytosis, the MCV is higher but due to absence of ovalocytes, the red cells have a less poikilocytosis and therefore the RBC cytogram shows a shift towards the zone of macrocytic cells but is less wide when compared to plots of megaloblastic marcocytosis (Fig 2)

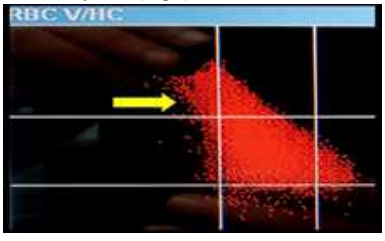

Fig No 2- RBC Scattergram In Non-megalobalstic Marcocytosis.

In dual nutritional deficiency or dimorphic anemia, the RBC cytogram is widely spread and exhibit two different populations in both the zones of microcytic and macrocytic cells.(Fig 3.)

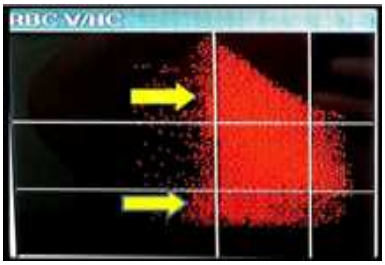

Fig No. 3 RBC Scattergram In Dimorphic Anemia.

In iron deficiency, the red cells showed shift to microcytic hypochromic area which is represented in the lower and left side of RBC cytogram. Patients of pancytopenia who were transfused with blood showed dual red cell populations. One population represents their own RBC and other of the transfused cells. (Fig 4.)

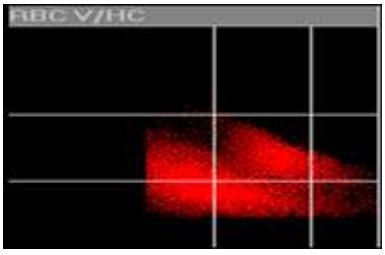

Fig No. 4 RBC Scattergram In Post Transfusion Iron Deficiency Anemia.

MCV and RBC Cytogram Findings: In present study, an MCV of <75fL was considered as microcytic, 75-95fL as normocytic and >95fL MCV values as macrocytic anemia[22]. Concordance of 92.18% was seen in between MCV values and RBC cytograms in cases of marcocytic anemia and that of 86.67% in microcytic anemia. However, only 21.95% concordance was seen in MCV ranging from 75 to 96fL. These discordant cases were mostly of dimorphic anemia where the numerical value of the MCV suggested a normocytic anemia which was not in concordance with the RBC scattergram findings. (Figure 5)

Deepthi et al[23] in their study concluded that MCV is not a reliable source for typing anemia especially dimorphic anemia as one type of anemia may mask the other and lead to discordant results as obtained in present study as well.

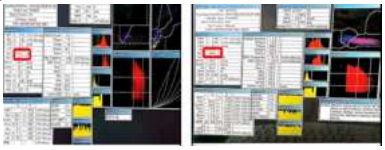

Fig No. 5 - Showing Discordance Between MCV And RBC Scattergram

Correlation Between PS and Cytogram Findings: In present study, there was concordance of 100% between PS and RBC cytogram findings for dimorphic hypochromic and normocytic hypochromic cases. There was 95.38% concordance for macrocytic hypochromic anemia.

Abnormal Scattergram Findings

In our study, 93.3% cases showed abnormal scattergram pattern. Maximum 62(51.67%) cases showed shift to left in neutrophil area followed by 38(31.67%) cases with widening of RBC ghost area and 30(25.21%) cases with widening of eosinophil area. Similar study by Modi et al[4] found that white blood cell scattergram of pancytopenic patients cases exhibited more than one abnormality. Cases of megaloblastic anemia cases showed four abnormalities. Most commonly observed was the shift of neutrophil area to left which is comparable to present study findings. Other findings were eosinophil area widening, ghost area widening and right shift of ghost area.

In present study, maximum cases of megaloblastic anemia showed shift to left in neutrophil area having statistical significance (p=0.00004). The "left shift of neutrophil area" in the WBC perox scattergram(Fig 6) represents a morphological shift to right on PS findings i.e. presence of hyper segmented neutrophils.

Fig No. 6- Scattergram Showing "Shift To Left Of Neutrophil Area" Megaloblastic Anemia

Ventura P et al[24] in their study stated that megaloblastic anemia showed frequent association with hemolysis. Vitamin B12 and folate are the common nutritional deficiencies in megaloblastic anemia which causes an alteration in homocysteine metabolism and results in

hyperhomocystenemia. This alteration is the possible mechanism for hemolysis in megaloblastic anemia.[24] In scattergram, these hemolysed rbc appear as "widening of rbc ghost area" which are represented in the platelet scattergram (Fig 7)

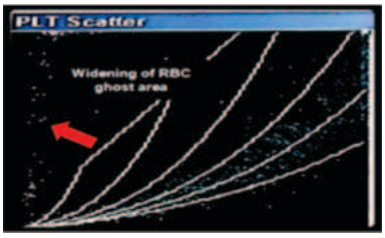

Fig No. 7 - Scattergram Showing "Widening Of RBC Ghost Area" In Megaloblastic Anemia

Sunethri P et al[25] studied the maturation of precursors of erythroid series in megaloblastic anemia and concluded that 78% cases showed an increase in the eosinophilic precursors without an obvious increase in the number of eosinophils on peripheral smear. An awareness to find eosinophilic precursor can be aided by observing the wbc scattergram which shows a "widening of eosinophilic areas" (Fig 8) in cases of megaloblastic anemia.

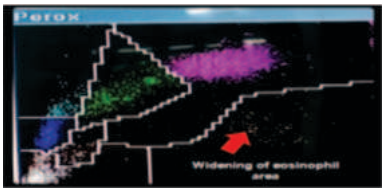

Fig No. 8: Scattergram Showing "Widening Of Eosinophil Area" In Megaloblastic Anemia

Cases diagnosed as dimorphic anemia showed WBC scattergram findings similar to that of megaloblastic anemia with only difference in the RBC cytogram findings where "dual population" pattern was seen.(Fig 3)

Cases reactive to infectious agent showed "wide neutrophil area" in cases of bacterial infection and a "rise in LUC (large unstained cell) area" in cases of dengue fever (Fig 9) The large unstained cells represented reactive lymphocytes in cases of dengue. Both the scattergram patterns of cases reactive to infection were statistically significant (p=0.0000)

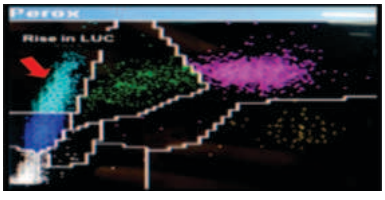

Fig No. 9 - Scattergram Showing "Rise In Luc Area" In Cases Reactive To Infection-dengue.

Cases of Subleukemic leukemia had statistically significant association (p=0.00001) with "rise in LUC area" and "dense blast area" (Figure 10).

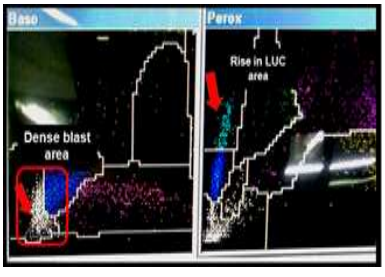

Fig No. 10 Showing "Dense Blast Area" And "Rise In Luc Area" In Acute Leukemia.

Myelodysplastic syndrome (MDS) cases also showed statistically significant(p=0.01) association with dense blast area however the blast areas were less dense in MDS as compared to leukemia (Fig 14)

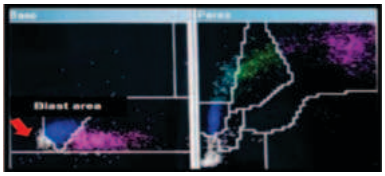

Fig No. 14- Scattergram Showing "blast Area" In Myelodysplastic Syndrome (MDS)

A study conducted by Jung MJ(9) et al[8] stated that LUCs give an idea about the nature of cells and helps to discriminate leukemic cell from the normal population of cells. Rabizadeh E. et al[26] in their study enumerated significance of paying attention to changes in LUC and blast areas in cases of acute leukemia. These scattergram changes provide precautionary clues for their diagnosis even before peripheral examination.

CONCLUSION

5 part hematology cell counter scattergram's graphical patterns in addition to the numerical parameters augment the analytical usefulness of automated data and provide necessary diagnostic evidences in the form of characteristic patterns even before peripheral smear examination. Patterns like shift to left in neutrophil area, rise in LUC area, dense blast area were proven statistically significant and can provide useful diagnostic clues. RBC scattergram patterns showed high concordance with the results of peripheral smear examination. Relying merely on the numerical values of RBC indices (MCV) showed considerable discordance as one anemia may mask the other providing misleading results. Here, scattergram patterns were proven useful for diagnosis.

Thus the present study helps to observe the patterns which provide help in segregating the causes of pancytopenia which require basic treatment from the ones which require extensive tertiary investigative procedures for its mere diagnosis.

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