STUDY OF WHITE BLOOD CELL SCATTERGRAM AS AN INDICATIVE PARAMETER FOR THE CAUSE OF PANCYTOPENIA

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ABSTRACT

BACKGROUND

Pancytopenia is a combination of anaemia, leucopoenia and thrombocytopenia with haemoglobin < 9 g/dl, total leukocyte count < 4000/cumm and platelet count < 1.4 lacks. Pancytopenia is a manifestation of many serious and life-threatening diseases. The major causes of pancytopenia in developing countries are megaloblastic anaemia, aplastic anaemia and hypersplenism. Pancytopenia is seen in various diseases like nutritional deficiency, haematological malignancy, aplastic anaemia and many others. In pancytopenia, many a times, differential count is difficult or impossible in peripheral smear because cell counts are too low. Whereas in cell counter large number of cells are examined, so WBC scatter gram can give a clue about the cause of pancytopenia. We wanted to evaluate the use of scatter grams to find the cause of pancytopenia.

METHODS

This is an observational study, in which all the patients with pancytopenia were included. After taking informed consent, a thorough clinical history was taken. The peripheral smear was made to examine the blood cell morphology and count. EDTA blood sample of pancytopenia cases were run in Mindray BC 5300 cell counter (Five part) and WBC scatter gram obtained. Simultaneously, peripheral smear examination was done. Bone marrow examination was also done as and when needed. Patterns of WBC scatter gram were correlated with cause of pancytopenia.

RESULTS

Total 80 cases were studied in which 38 cases are of megaloblastic anaemia, 26 cases were of dimorphic anaemia, followed by 4, 5 and 3 cases of hypoplastic anaemia, aplastic anaemia and reactive to infection respectively and 1 case each of AML, MDS (refractory anaemia with excess blast - 1). 2 cases remain undiagnosed. Deficiency anaemia is still the most common cause of pancytopenia and these can be very well diagnosed by scatter gram. Megaloblastic anaemia shows specific pattern in scatter gram like shifting of neutrophil area to left, widening of ghost area and widening of eosinophil area. Dimorphic anaemia also shows features of megaloblastic anaemia along with right shift of ghost area.

CONCLUSIONS

It is well known that causes of pancytopenia ranges widely from the ones requiring basic treatment modalities to the ones desiring extensive high-end battery of advanced investigative procedures & vigorous treatment having grave prognosis. With the help of scatter gram, we are capable of segregating the causes of pancytopenia requiring basic treatment protocols from the ones requiring extensive tertiary investigative procedures for its mere diagnosis.

KEY WORDS

Pancytopenia, Scatter Gram, Cell Counter, Deficiency Anaemias

HOW TO CITE THIS ARTICLE: Modi V, Choudhary R, Nigam RK. Study of white blood cell scattergram as an indicative parameter for the cause of pancytopenia. J. Evolution Med. Dent. Sci. 2019;8(29):2333-2337, DOI: 10.14260/jemds/2019/509

BACKGROUND

Pancytopenia is a combination of anaemia, leucopoenia and thrombocytopenia with haemoglobin < 9 g/dl, total leukocyte count < 4000/ cu mm and platelet count < 1.4 lacks.[1] Pancytopenia is a manifestation of many serious and life-threatening diseases. The major causes of pancytopenia in developing countries are megaloblastic anaemia, aplastic anaemia and hypersplenism.[1]

Peripheral smear evaluation may reveal important information regarding aetiology but in severe pancytopenia cell count may be very low to make differential count impossible or very difficult.[2] To make peripheral smear we spread a small drop of blood and examination done at junction of body and tail of smear. So, a very little fraction of blood is examined. Whereas in cell counters around 20 microliters blood is examined.[3] So we get large number of cells as compared to peripheral smear. Cell counter gives us counter specific parameters, histograms and scatter gram. Here we focus on scatter gram where cells are plotted according to cell size, granularity and nuclear shape and size. Pancytopenia is blood picture of various diseases like nutritional deficiency[4]-[6], haematological malignancy, aplastic anaemia[7]-[10] and many others. In pancytopenia at many times differential count is difficult or impossible in peripheral smear because cell counts are too low. Whereas in cell counter large number of cells are examined, so WBC scatter gram can give a clue about the cause of pancytopenia.

'Financial or Other Competing Interest': None.
Submission 24-04-2019, Peer Review 01-07-2019,
Acceptance 09-07-2019, Published 22-07-2019.
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DOI: 10.14260/jemds/2019/509

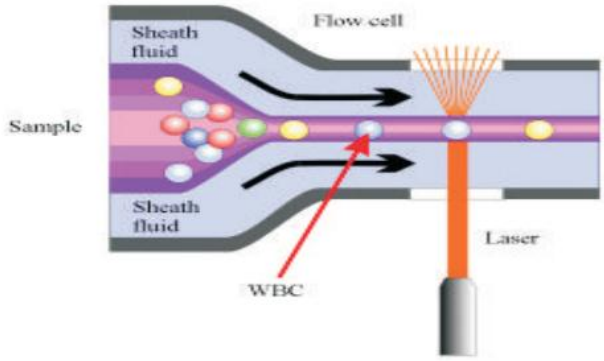


Figure 1. Principle of Flowcytometry and Fluidics

Source: Internet- preface to clinical case study for Mindray haematology analyser.

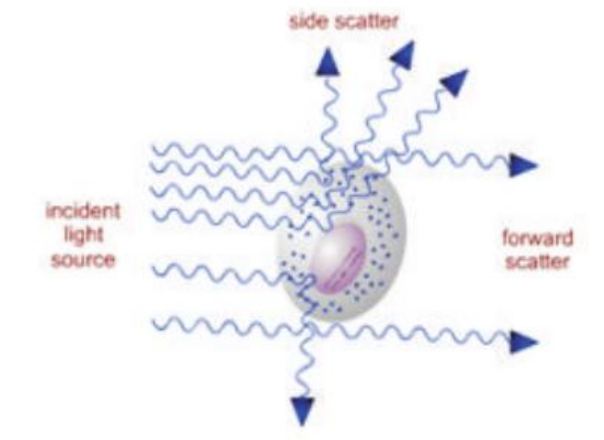


Figure 2. Laser Scatter

Source: Internet - preface to clinical case study for mindray haematology analyser.

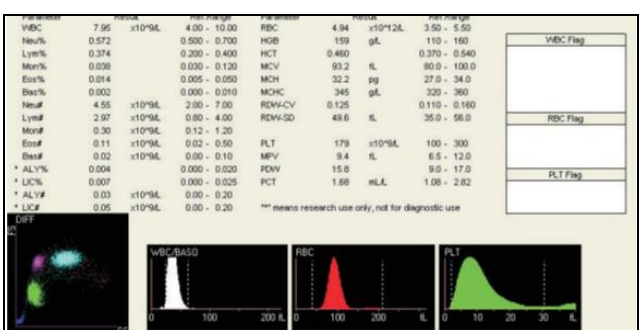
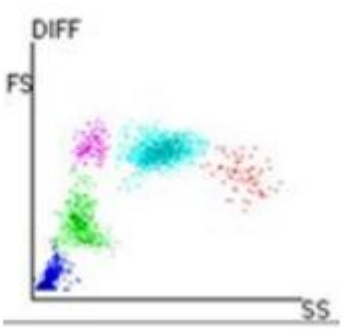
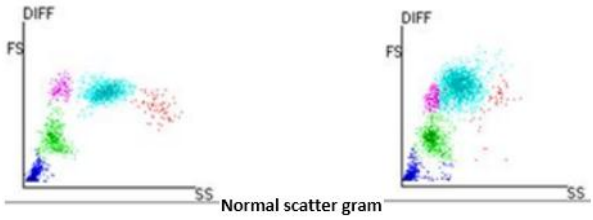


Figure 3. Normal Scatter Gram with Normal Histograms and all Parameters within Normal Range

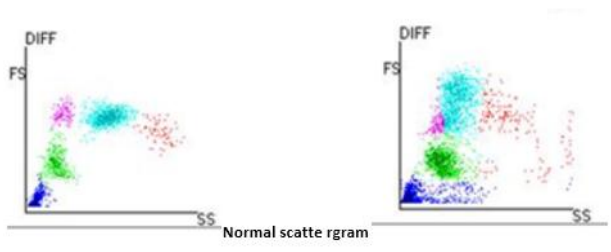


Graph 1. Normal Scatter Gram (Scatter Plot). Forward Scatter (FS) Represents Cell Volume and Side Scatter (SS) Represents Cell Granularity

Megaloblastic Anaemia



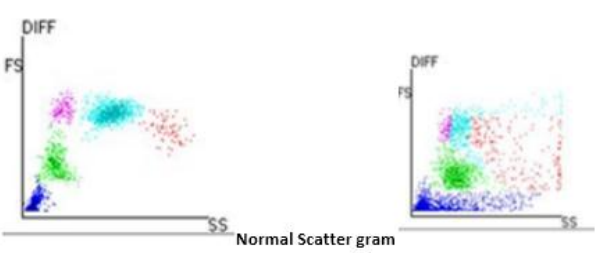
Graph 2. Shift of Neutrophil Area to Left as Compare to Normal Scatter Gram



Graph 3. Widening of Ghost Area, Widening of Eosinophil Area and Rightward Shift of Ghost Area

Dimorphic anaemia showed all the abnormalities found in megaloblastic anaemia along with dual population of RBCs in RBC histograms. In dimorphic anaemia rightward shift of ghost area is more common.

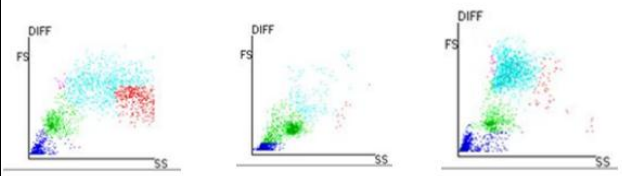
Dimorphic Anaemia



Graph 4. Shift of Neutrophil Area to Left, Widening of Eosinophil Area, Rightward Shift of Ghost Area

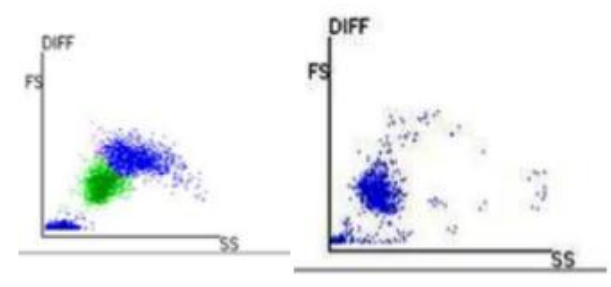
Reactive to infection cases show widening of neutrophil area in scatter gram. AML showed downward shift of neutrophil area with widening.

Reactive to Infection

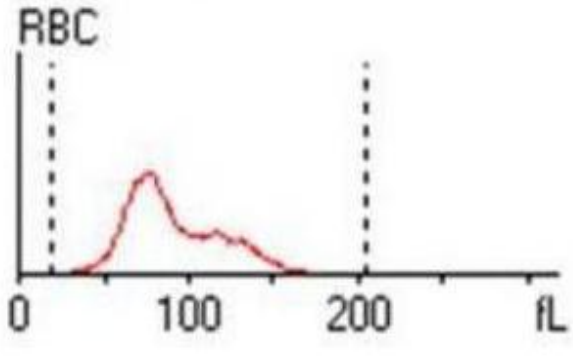


Graph 5. Wide Neutrophil and Eosinophil Area

Bluish discolouration of neutrophil area and only ghost area were also found in some scatter grams which was unspecific.



Graph 6. Bluish Discoloration and Only Ghost Area



Graph 7. Dual Population in RBC Histogram

Cause of Pancytopenia	Number of Cases (80)	Percentage (%)
Megaloblastic anaemia	38	47.5 %
Dimorphic anaemia	26	32.5 %
Aplastic	5	6.25 %
Hypoplastic anaemia	4	5 %
AML	1	1.25 %
MDS (RAEB-1)	1	1.25 %
Reactive to infection	3	3.75 %
Undiagnosed	2	2.5 %
Total	**80**	**100 %**

Table 1. Different Causes of Pancytopenia

Abnormality of Scatter Gram	Cause of Pancytopenia Showing Abnormality	Number of Cases Showing Abnormality
1. Shifting of neutrophil area to left.	Megaloblastic anaemia, dimorphic anaemia	60
2. Widening of RBC ghost area	Megaloblastic anaemia	35
3. Widening of eosinophil area	Dimorphic anaemia, Megaloblastic anaemia	48
4. Rightward shift of RBC ghost area	Dimorphic anaemia, megaloblastic anaemia	23
5. Dual population of RBCs in histogram	Dimorphic anaemia	25
6. Normal pattern	Aplastic	3
7. Only ghost area	Hypoplastic anaemia, Aplastic	4
8. Bluish discolouration of neutrophil area	Dimorphic anaemia, megaloblastic anaemia Hypoplastic anaemia, undiagnosed case	6
9. Widening of neutrophil area	Reactive to infection	3

Table 2. Different Abnormalities in WBC Scatter Gram in Pancytopenia Cases

Aim of The Study

Evaluate the use of scatter grams to find the cause of pancytopenia.

METHODS

This is an observational study, in which all the patients with pancytopenia were included. After taking informed consent, a thorough clinical history was taken. The peripheral smear was made to examine the blood cell morphology and count. Simultaneously EDTA blood sample was also collected for manual complete blood count and to run in cell counter. Scatter gram analysis was also done to find a specific pattern of scatter gram. Some selected cases having very low counts were taken for bone marrow examination. We correlated the cause of pancytopenia- final diagnosis made with pattern of scatter grams. In our study we used Mindray BC 5300 (5 part) cell counter. This gives 27 parameters 3 histograms and 1 scatter gram.

Inclusion Criteria

All patients of pancytopenia referred to department of pathology for various laboratory tests from 1 st of June 2018 to 31st January 2019.

Exclusion Criteria

Patients having pancytopenia whose follow up not possible (OPD Patients).

Principle of Cell Counter

Mindray BC 5300 Cell counter works on the principle of Flowcytometry.[3]

Flowcytometry

The basic principle of flowcytometry is the passage of cells in single cell line in path of a laser, cells deflects laser which is detected by sensors.

In Mindray BC 5300 cells are injected into a flow cell which is located in the optical path of a light source, usually a laser; Surrounded with sheath flow, the blood cell pass through the centre of flow cell in a single column at a fast speed. There are two parts one is fluidics and second is optical system.

Fluidics

Hydrodynamic Focusing Principle

The sample fluid flows in a central core that does not mix with the sheath fluid. Only one cell or particle can pass through the laser beam at a given moment.

Optical System

After the cell delivery system, the need is to excite the cells using a Laser. When a light intersects a laser beam at the so called 'Interrogation Point' Light scattering occurs. When light from a laser interrogates a cell, that cell scatters light in all directions.

Optics- Laser Scatter

Light scattering occurs when a particle deflects laser light. The extent to which this occurs depends on the physical properties of the particle.[2]

Forward Scatter (FSC)

Light that is scattered in the forward direction (Along the same axis the laser is travelling) is detected in the Forward Scatter Channel. Intensity of this signal has been attributed to cell size.

Side Scatter (SSC)

Laser light that is scattered at 90 degrees to the axis of the laser path is detected in the Side Scatter Channel. The intensity of this signal is proportional to the amount of cytosolic structure in the cell (e g. granularity, internal complexity, etc.)

Sky blue coloured dots are neutrophils and that is their normal area in scatter plot (Scatter gram). Pink colour dots are monocytes and that is their normal area in plot. Green colour dots are lymphocytes that is their normal area in scatter plot. Red colour dots are eosinophil and their normal area in scatter plot. Blue colour lowest dots are ghost area which represents RBCs. Fragmented Cell platelets and debris.

Normal scatter gram appearance in this scatter gram, the WBC sub-populations are well differentiated from each other and aggregate within expected. The WBC/BASO, RBC and PLT histograms are also normal and there is no flag. All the parameters are in normal range.

Statistical Analysis

The data was collected and analysed using standard statistical chi – square test, $P < 0.05$ statistically significant. Data was entered in Microsoft excel and analysis was done using SPSS version 22

RESULTS

In our study the megaloblastic anaemia is still the most common cause of pancytopenia. Total 80 case were studied in which 38 case are of megaloblastic anaemia, 26 cases of dimorphic anaemia, followed by 4, 5 and 3 cases of hypoplastic anaemia, aplastic anaemia and reactive to infection respectively and 1 case each of AML, MDS (Refractory anaemia with excess blast – 1). 2 cases remain undiagnosed. Deficiency anaemia are still the most common cause of pancytopenia and these can be very well diagnosed by scatter gram. In selected cases where pancytopenia is more severe bone marrow examination was also done. 2 cases remain undiagnosed because they refused for bone marrow examination and showed atypical scatter gram and other counter parameters. WBC scatter gram of pancytopenia cases showed more than one abnormality. Megaloblastic anaemia cases show mainly four abnormalities, most common was shifting of neutrophil area to left, other was widening of ghost area, widening of eosinophil area and right shift of ghost area.

DISCUSSION

Sunita Sharma et al 2012 studied WBC scatter grams to find ability of scatter gram to provide significant clues about malaria. They found Greying of eosinophil and neutrophil groups, two eosinophil population, Overlapping of neutrophil and eosinophil groups and two neutrophil population like abnormalities in malaria positive cases. They used XT2000i auto haematology analyser in their study. [11]

Many other studies also have been conducted all over world using the cell counters in malaria detection.[12]-[15]

In our study we focused only on WBC scatter grams of pancytopenia cases. In which the most common abnormalities found are shifting of Neutrophil area to left, Widening of RBC ghost area, Widening of eosinophil and Rightward shift of RBC ghost area. All these abnormalities were correlated with deficiency anaemia.

CONCLUSIONS

It is well known that causes of pancytopenia range widely from the ones requiring basic treatment to complicated treatments which may have grave prognosis.[16]-[24] With the use of scatter gram we can easily segregate conditions which require simple treatments that are available in majority of health setups like community health centres there by avoiding high cost investigations. Therefore, usage of scatter grams will prove immensely helpful in developing countries like India to provide better health care with limited financial and human resources.

ACKNOWLEDGEMENT

Dr. Renni Malik, Professor and Head of Department of Pathology, Gandhi Medical College, Bhopal. Dr. Suhas Kothari, In-Charge, Central Pathology Lab, Gandhi Medical College, Bhopal. Dr. Pramila Jain, In-Charge, Central Pathology Lab, Gandhi Medical College, Bhopal. Dr. Atul Shrivastava, Head, Cancer Registry, Department of Pathology, Gandhi Medical College, Bhopal. Dr. Abhinav Junwal, Resident Medical Officer, Department of Pathology, Gandhi Medical College, Bhopal.

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