Clinico-hematological profile and etiological spectrum of pancytopenia: An institutional study

Geetanjali N1, Subhashish Das2*

1Senior Resident, 2Professor, Dept. of Pathology, Sri Devraj Urs Medical College, Kolar, Karnataka, India

*Corresponding Author: Subhashish Das
Email: daspathology@gmail.com

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Abstract

Introduction: Pancytopenia is a common hematological problem characterized by anemia, leucopenia and thrombocytopenia. It is a striking feature of many serious and life threatening illnesses. The disease pattern varies in different population groups, in age pattern, nutritional status and prevalence of infective disorder. Present study was conducted to assess the etiology, clinical profile and bone marrow morphology of pancytopenia.

Objectives: 1. To study the clinical presentations and hematological parameters in patients with pancytopenia. 2. To study the morphological pattern in bone marrow in patients with pancytopenia.

Materials and Methods: A two year study was conducted in department of Pathology, Sri Devaraj Urs medical college, Kolar, Karnataka. Total 70 pancytopenia patients were studied to determine their presenting symptoms and signs, peripheral blood smear study and bone marrow morphology.

Results: Bone marrow study showed 48.5% hypercellular marrow, 32.8% normocellular and 18.7% hypocellular marrow. Females (55.7%) were affected more than males (44.2%) and the commonest cause for pancytopenia was megaloblastic anemia (51.4%), followed by iron deficiency anemia (37.1%), viral infections (4.2%), leukemia (1.4%), MDS (2.8%), pure red cell aplasia (1.4%), hereditary elliptocytosis (1.4%).

Conclusion: Large number of patients with pancytopenia had reversible etiology. Hence complete work up including clinical details with hematological examination along with bone marrow study will lead to early and proper diagnosis of case followed by proper treatment.

Keywords: Pancytopenia, Megaloblastic anemia.

Introduction

Pancytopenia is not a disease entity, but triads of findings that may result from a number of diseases processes—primarily or secondarily involving the bone marrow. The presenting symptoms are often attributable either to the anemia or thrombocytopenia. Anemia leads to fatigue, dyspnea and cardiac symptoms. Thrombocytopenia leads to bruising and mucosal bleeding. Neutropenia leads to leucopenia seen in the subsequent course of the disorder.

Many hematopoietic and non-hematopoietic conditions manifest with features of pancytopenia. 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 aplasias and leukemias.

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 the infective disorder.

Materials and Methods

Patients diagnosed to have pancytopenia based on hematological criteria and requiring bone marrow examination was studied at R L Jalappa Hospital and Research Centre. Seventy patients were selected who have pancytopenia on peripheral blood smear based on hematological criteria of hemoglobin less than or equal to 10 gm /dl, Total leucocyte count less than or equal to 4000/cumm, Platelets less than or equal to 1,00,000/cumm were selected. Patients on myelotoxic chemotherapy were excluded from the study.

The study included analysis of 70 prospective cases in Central laboratory of R L Jalappa Hospital and Research Centre was studied from 2013 to 2015. Hence it is a prospective study.

Patient details such as age, sex, drug history, occupation, past medical illness, general examination of all the identified cases of pancytopenia were done as per the proforma.

2 ml of EDTA (Ethylene-diamine-tetra-acetic acid) anti-coagulated blood was collected and processed through a hematology analyzer and parameters like complete blood cell count, total leucocyte count, differential count, Mean corpuscular volume (MCV), Mean corpuscular hemoglobin concentration (MCHC), Mean corpuscular hemoglobin (MCH) Packed cell volume (PCV) were evaluated by Beckman Coulter based on the hematological criteria. Internal Quality control is run every day in hematology department. Corrective measures are taken if values go beyond ± 2 SD. External Quality control is also performed as our hematology department registered with All India institute of medical sciences, New Delhi.

Peripheral smear was studied by Leishman stain for morphological detail.

Out of seventy patients, bone marrow aspiration was performed in 67 cases, three cases where the aspiration was unsuccessful biopsy was performed.
After taking informed consent from the patient or the guardian bone marrow aspiration or biopsy was done on posterior iliac crest of the patients under aseptic precautions.

Bone marrow smears were made and stained with May-Grünwald Geimsa (MGG) Stain.

**Staining Procedure**

Logistics and materials:
1. Leishman stain
2. Buffered distilled water (pH 6.8-7.2)
3. Timer
4. Slide
5. EDTA blood sample

**Smear Preparation**

1. Smear was covered with Leishman’s stain
2. It was allowed to stand for 1-2 minutes.
3. Without removing the stain, double the amount of buffered distilled water was added.
4. Allowed it to stand for 7 minutes.
5. Slide was flooded with tap water.
6. Back of the slide was washed with soap and water
7. It was air dried in a tilted / upright position

A Well stained film had the following features:
1. The nuclei of leucocytes was purple
2. Neutrophilic granules – tan in colour
3. Eosinophilic granules – red orange in colour
4. Basophil – dark purple granules
5. Platelets – had dark lilac granules
6. Cytoplasm of lymphocytes – light blue
7. RBC’s – pink colour

**Bone Marrow Aspiration**

This was done in sixty seven patients under proper sterile precautions. First an informed consent was obtained. Then the patient was positioned properly and local anaesthesia was given after a test dose. A Salah’s needle was used to aspirate material from right posterior iliac crest in majority of cases. Needle and the stillette were placed in position and the cap was closed. After piercing the skin and the subcutaneous tissue, the periosteum and cortex were pierced in a clockwise rotatory motion. Once in the marrow cavity, the stillette was removed and 0.2-0.3 ml of marrow fluid was aspirated with a sterile disposable 20ml syringe. The aspirate was then transferred to a watch glass filled with 5ml of sodium citrate and was mixed gently. Then with the help of a needle, marrow particles were transferred to the slides and smears were made and allowed to dry. Slides were then stained with Leishman’s stain and MGG. The bone marrow aspirate smear is a preparation designed to spread the cellular material of the marrow so that Romanowsky’s stain can reveal essential cellular details.

**Staining Procedure**

1. May Grunwald Geimsa (MGG)
2. Buffered distilled water (pH 6.8-7.2)
3. Slide

**Bone marrow aspirate sample**

**Smear Preparation**

1. Smear was covered with Geimsa (MGG) stain
2. It was allowed to stand for 1 minute.
3. Without removing the stain, double the amount of buffered distilled water was added.
4. Allowed it to stand for 15-20 minutes.
5. Slide was flooded with tap water.
6. It was air dried in a tilted / upright position

The bone marrow aspirate was evaluated as follows:

1. A low power scan of the bone marrow aspirate was done to see if the material obtained was satisfactory and also to assess its cellularity.
2. A high power view and oil immersion lens were used to determine the distribution and morphology of cell types.

The smears were assessed as per the following format:

- Cellularity of the fragments
- Erythropoiesis - cellularity, maturation pattern and any cytological abnormalities
- Myelopoiesis - cellularity, maturation pattern and any abnormalities
- M: E ratio – to count 500 cells
- Megakaryopoiesis – number, morphology, presence of immature forms
- Lymphocytes
- Plasma cells
- Parasites / abnormal cells / Granulomas / storage cells

**Bone Marrow Biopsy**

This was done in three cases where aspiration was unsuccessful. Following aspiration, another needle (Trephine) was pushed into the cavity at the same site and the stillette was removed and then pushed further into the cavity by clockwise rotating movement for about 1-1.5 cm. This procedure captures the marrow core sample within the needle. The needle was then withdrawn in ant clockwise rotating motion. Then the stillette was inserted and biopsy taken out. Imprints were taken on a glass slide. The specimen was fixed in 10% formalin overnight and decalcified with EDTA. Then it was processed similar to histopathological sample and H & E sections were studied.

The microscopic examination of fixed, embedded sections of the bone marrow tissue as represented by biopsy is best for determining the overall cellularity and presence of infiltrates. A much larger percentage of the bone marrow can be scanned in the biopsy specimens compared to the bone marrow aspirate. Touch imprint preparations from the biopsy material are also useful for morphology.

**Trephine Biopsy Interpretation - H & E sections were studied as follows**

**Adequacy of the Biopsy**

The ideal specimen was 1-2 cm in length, and was not distorted and had at least 5 well preserved intertrabecular marrow spaces for interpretation.

1. Cellularity - Normocellular/ hypocellular/ hypercellular
2. Erythropoiesis - cellularity and distribution
3. Myelopoiesis - cellularity and distribution
4. Megakaryopoiesis - number, morphology, immature forms
5. Marrow fibrosis
6. Presence of abnormal cells / parasites / granulomas
7. Miscellaneous – amyloidosis, necrosis, gelatinous transformations etc.

Statistical Analysis
Frequencies: The frequencies procedure provides statistics and graphical display that are useful for describing many types of variables. For a frequency report and bar chart, we can arrange the distinct values in ascending or descending order or order the categories by their frequencies. The frequencies report can be suppressed when a variable has many distinct values. We can label charts with frequencies or percentages.

Chi square test: Continuous data was summarized in percentage. The categorical variables were compared by chi-square test. Pearson’s correlation analysis was used to assess association between the variables. A p value <0.05 was considered statistically significant. All analyses were performed on SPSS Software (22 version).

Results
Seventy patients with a hematological diagnosis of pancytopenia were studied during the period December 2015 to December 2017 in the Department of Pathology, Sri Devaraj Urs Medical College, Tamaka, Kolar.

Out of seventy patients, bone marrow aspiration was done in 67 cases, in 3 cases where aspiration was unsuccessful biopsy was confirmed. The following data were recorded and analyzed. Primary haematological procedures were carried out in all 70 patients, in which haematological parameters and peripheral smears and bone marrow were examined in detail.

1. The following parameters are discussed below:
2. Age distribution and Sex distribution
3. Presenting symptoms and signs
4. Hematology data – Hemoglobin, Leucocyte count, platelet count, MCV.
5. Peripheral blood smear findings
6. Bone marrow cellularity
7. Erythroid series findings
8. Myeloid series findings
9. Megakaryocyte findings

As shown in the table 1, most of the patients were in the age group of 51-60yrs seen in 16 (23.9%) and the least occurrence was seen in the age group of below 20yrs and 41-50 years (13%). The sex distribution of pancytopenia showed a female preponderance. The female to male ratio was 1.25:1.

As shown in the table 2 fever 47(67.1%) was the most common symptom and the least common symptom was breathlessness 2 (2.8%) and altered sensorium1 (1.4%).

As shown in the table 3 (34.2%) showed maximum hemoglobin range of 3.1-5 gm/dl and minimum hemoglobin range was 1-3 gm/dl seen in 10(14.9%) of the cases.

37(55.2%) of the cases showed maximum Leucocyte range of 3100-4000/cumm and minimum leucocyte range was 500-1000/cumm seen in 3(4.2%) of the cases. 23(34.3%) cases showed maximum platelet count range was 76,000-1,00,00 and minimum platelet count range was seen in 10(14.9%) of the cases.

As shown in the table 4, 31 (44.2%) of patients had Dimorphic anemic blood picture, and 8 (11.4%) of the cases had microcytic hypochromic anemia followed by one case each of acute leukemia and hereditary elliptocytosis.

Bone marrow aspirate in the present study of pancytopenia showed the following types of cellularity: a) Hypocellularity -18.7%, b) Normocellularity – 32.8%, c) Hypercellularity- 48.5%.

As shown in the table 6 megaloblastic maturation was seen in 23 (32.8%) of the patients and 17(24.2%) showed dimorphic maturation pattern (both micronormoblastic and megaloblastic maturation).

Pancytopenia with Myelodysplastic Syndrome
In the present study, two cases of myelodysplastic syndrome was observed. One case was of 40 year old male with fever and generalized weakness. Total count was 1200/cumm, hemoglobin was 7.3 gm/dl, mean corpuscular volume was 79fl, and platelet count was 78,000/cumm. Peripheral blood smear erythrocytes showed normocytes with hypochromasia, white blood cells was decreased in count, platelets was also reduced in number. Bone marrow aspiration was done and revealed hypercellular marrow with dyserythropoiesis and M:E ratio-10:1. Erythroid series showed megaloblastic maturation, also seen were certain dyserythropoietic features like nuclear fragmentation, budding multiple nuclei and hyperlobulation. Myeloid series were reduced in number with few atypical cells seen constituting around 9% of blasts. Megakaryocytes showed giant forms and hypolobulated forms. Hence, a diagnosis of myelodysplastic syndrome RAEB-1 was made.

Another case was of 60 year old male with generalized weakness and splenomegaly. Total count was 1000/cumm, hemoglobin was 6 gm/dl, mean corpuscular volume was 86fl, platelet count was 48,000/cumm. Peripheral blood smear erythrocytes showed normocytes with hypochromasia, white blood cells were decreased in count and platelets were reduced in number.

Bone marrow aspiration was unsuccessful hence bone marrow biopsy was performed under aseptic precautions which revealed hyper cellular marrow, M: E ratio could not be assessed. Erythroid series showed megaloblastic maturation with dyserythropoiesis. Myeloid series showed immature granulocytes. Megakaryocytes were increased in number and showed dysplastic changes, hypolobated forms were also seen. Additionally eosinophils and plasma cells were also seen. Hence a diagnosis of Myelodysplastic marrow was confirmed.

Pancytopenia with Acute Leukemia
In the present study, one case of acute leukemia was observed presenting with pancytopenia. A 24 year old...
female with generalized weakness, fever and cough. On examination there was no hepatosplenomegaly. Total count was 4000/cumm, hemoglobin was 4.3 gm/dl, mean corpuscular volume was 105 fl, and platelet count was 16,000/cumm. Peripheral blood smear erythrocytes showing macrocytosis, white blood cells showed 20% blasts which were large and mononcoid with Auer rods which were round to oval with raised N:C ratio, disperse chromatin and one to two prominent nucleoli, platelet counts were decreased. Bone marrow aspiration revealed hyper cellular marrow with M: E ratio -5:1. Erythroid series showed megaloblastic maturation with few binucleate forms, myeloid series were also reduced in number with presence of immature cells (blasts) having high N: C ratio, coarse chromatin and 1-2 prominent nucleoli. Blasts constituted more than 25%. Megakaryocytes were reduced in number.

### Pancytopenia with Pure Red Cell Aplasia

In the present study, one of the cases of pure red cell aplasia was observed which presented with pancytopenia. A 62 year old female who presented with fever and generalized weakness, on examination showed pallor, with no signs of lymphadenopathy or hepatosplenomegaly. Total leucocyte count was 4000/cumm, Hemoglobin was 3 gm/dl, mean corpuscular volume was 51 fl, and platelet count was 28,000/cumm. Peripheral blood smear showed microcytic hypochromic blood picture. Bone marrow aspiration revealed hypocellular bone marrow and M: E ratio was found to be 2:1. Erythroid series was reduced showing few micronormoblastic maturation and also seen were bizarre (Red Blood Cell) RBC lineage, Myeloid series showed dysplastic changes and showed increased mast cells. Megakaryocytes were normal in morphology. Hence, the diagnosis of pure red cell aplasia was made based on the bone marrow aspiration findings observed.

### Pancytopenia with Hereditary Elliptocytosis

In the present study, one case of hereditary elliptocytosis was observed. A fifty year old male with family history of hereditary elliptocytosis presented with fever, cold, bodyache and malena. Total count was 3,800/cumm, hemoglobin was 9 gm/dl, mean corpuscular volume was 92 fl, and platelet count was 60,000/cumm. Peripheral blood smear showed severe anisopoikilocytosis with cigar shaped red blood cells – Elliptocytosis, white blood cells were normal in morphology, No atypical cells were observed in the smear studied. Bone marrow was hypocellular with M: E ratio was 1:2. Erythroid series showed erythroid hyperplasia with normoblastic maturation, myeloid series and megakaryocytes were normal in number and morphology.

### Statistical Analysis

Continuous data was summarized in percentage. The categorical variables were compared by chi-square test. Pearson’s correlation analysis was used to assess association between the variables. A p value <0.05 was considered statistically significant.
malignancy, granulomatous disease, multiple myeloma.\textsuperscript{11} P Shailaja et al showed that megaloblastic anemia in 38.9% of the patients was the commonest cause of pancytopenia.\textsuperscript{12} It was also observed that 7 patients who were on anti-tubercular treatment showed pancytopenia as anti-tubercular drugs are known to reduce serum folate levels in megaloblastic anemia.

M A Azad et al showed megaloblastic Anemia in 28% of patients was the commonest cause of pancytopenia followed by aplastic Anemia seen in 20% of the patients.\textsuperscript{13} The other causes included Myelodysplastic syndrome, acute myeloid leukemia, Non-Hodgkin’s lymphoma, and SLE, HIV/AIDS, Hypersplenism and Hemophagocytic syndrome. The commonest cause of pancytopenia, reported from various studies throughout the world has been aplastic anemia followed by megaloblastic anemia.

This is in sharp contrast with the results of the present study where the commonest cause of pancytopenia was megaloblastic anemia followed by nutritional anemia. This thus reflects the higher prevalence of mixed nutritional anemia in our population. In the present study, megaloblastic anemia was observed 36(51.4%) and the second most common cause was found to be nutritional anemia seen in 26(37.1%).The possible explanation for this would be due to low socioeconomic status, food habits, superstitious beliefs and due to lower literacy rates patients are less compliant towards treatment.

Mixed nutritional anemia is well recognised as a common etiological factor causing pancytopenia. Iron deficiency anemia also results as a nutritional deficiency. Hence, mixed deficiency anemias due to deficiency of iron, vitamin B12 and folate can cause peripheral cytopenia.

In the study done by Mobina et al on 392 cases of pancytopenia, (11.2%) were found to be due to mixed deficiency anemias.\textsuperscript{76} in the study done by Shazia Menon, mixed deficiency was seen in 20 cases (8.69%).\textsuperscript{77}

In the present study mixed deficiency was seen in 26(37.1%) patients as shown in the above table 40, the possible explanation may be that, majority of the cases present with anemia rather than pancytopenia and are diagnosed on peripheral smear examination and treated as outpatients. This percentage is much lower than the expected because 60-80% of world population is affected by iron deficiency anemia which is the most common preventable nutritional deficiency in the world.

Vitamin B12, folic acid and iron studies were not done as it was not affordable by the patients

In a study done by Kripal Das Makheja et al megaloblastic anemia was seen in the age group of 41-50 years and had a male to female ratio of 1.2:1.\textsuperscript{14} In a study done by Senjuthi Dasgupta et al the common age group was 5-15 years the male to female ratio was 1.7:1.\textsuperscript{15}

In the present study, age ranged from 51-60 years, which was found to have a statistically insignificant association (p=0.151); table 33 and the female to male ratio is 1.2:1, with a female preponderance in megaloblastic anemia; which was had a statistically significant association (p=0.018). This is due to lack of availability of care, nutritional education in the elderly age group. Female are more affected due to social neglect and lack of education.

The commonest affected age group in mixed nutritional anemia was more than 60 years. There was a female preponderance and female to male ratio was 2.25:1.Majority of the patients presented with fever, generalised weakness and bleeding tendencies. Pallor was the most common sign seen in 30 (42.8%) of the cases among this study group. This is due to loss of blood through menstruation in addition to nutritional deficiencies.

Khodke et al. and Tilak et al. reported one case of AML causing pancytopenia,\textsuperscript{5} the age ranged from 3-69 years and 5-70 years respectively. In the study done by Jha et al., acute leukemia alone constituted (90.6%) of all the hematological malignancies. It accounted for (19.5%) of total cases of pancytopenia. Age ranged from 2-75 years with a male to female ratio of 1.9:1.\textsuperscript{16}

In the present study, the malignant diseases accounted for 1.4% of pancytopenia. One case of acute myeloid leukemia (AML M4) was encountered which was of 24 year old male presenting with peripheral cytopenia.

**Myelodysplastic Syndrome**

Pancytopenia is a common finding in Myelodysplastic syndrome, but least common finding encountered in patients with MDS as compared to mono and bicaetyopnia. In a study of 31 patients of MDS by Kini J et al., bicaetyopnia was the commonest finding.\textsuperscript{17} Greenberg et al studied 816 patients and observed pancytopenia in 15% of the patients.\textsuperscript{18}

Kini J et al observed that 31 patients in the study population were in the age group 40-70 years.\textsuperscript{18} In a study of 118 patients with MDS by Juneja SK et al the age ranged from 48-95 years.\textsuperscript{19}

In the present study, the age group in MDS patients was observed to be between 40 -60 years.

**Table 1: Age and sex distribution in cases of pancytopenia**

| Age (in years) | Gender | Total | Percentage (in %) |
|---------------|--------|-------|-------------------|
| <20           | 3      | 6     | 12.9              |
| 20-30         | 5      | 9     | 20                |
| 31-40         | 5      | 7     | 12.1              |
| 41-50         | 3      | 6     | 12.9              |
| 51-60         | 12     | 4     | 22.9              |
| >60           | 3      | 7     | 14.3              |
| Total         | 31     | 39    | 100               |

**Table 2: Distribution of presenting symptoms in pancytopenia patients**

| Symptoms          | Total | Percentage (in %) |
|-------------------|-------|-------------------|
| Generalised weakness | 37    | 52.8              |
| Fever             | 47    | 67.1              |
| Easy fatigability  | 33    | 47.1              |
| Pain abdomen       | 8     | 11.4              |
| Breathlessness     | 2     | 2.8               |
Table 3: Range of hematological parameters in pancytopenia

| Hemoglobin (in gm/dl) | Number of Patients | Percentage (in %) |
|-----------------------|--------------------|------------------|
| 1-3                   | 10                 | 14.9             |
| 3.1-5                 | 24                 | 34.2             |
| 5.1-7                 | 14                 | 20               |
| 7.1-10                | 22                 | 31.4             |
| TOTAL                 | 70                 | 100              |

Table 4: Distribution of peripheral blood smear findings in pancytopenia

| PBS                        | Male | Female | Total | Percentage (in %) |
|----------------------------|------|--------|-------|-------------------|
| Dimorphic Anemia           | 13   | 18     | 31    | 44.2              |
| Normocytic Anemia          | 8    | 9      | 17    | 24.3              |
| Macrocytic Anemia          | 5    | 7      | 12    | 17.1              |
| Microcytic hypochromic Anemia | 3  | 5      | 8     | 11.4              |
| Acute myeloid leukemia – M4 | 1   | 0      | 1     | 1.4               |
| Hereditary Elliptocytosis   | 1    | 0      | 1     | 1.4               |
| **Total**                  | **31**| **39**| **70**| **100**           |

Table 6: Erythroid series findings in pancytopenia

| Erythroid series | Total | Percentage (in %) |
|------------------|-------|-------------------|
| Micronormoblastic maturation/megaloblastic maturation | 17 | 24.2 |
| Megaloblastic Maturation | 23 | 32.8 |
| Micronormoblastic maturation | 12 | 17.4 |
| Micronormoblastic maturation | 18 | 25.7 |
| **Total** | **70** | **100** |

Distribution of Symptoms and signs in Pancytopenia

Studies done by B Deepak et al suggests that generalized weakness was the commonest symptom and pallor was the most predominant sign in megaloblastic anemia. Taj Ali Khan et al in observed that the common clinical presentations were pallor, fever weakness, bleeding manifestations splenomegaly and gastrointestinal symptoms. In the present study, 27(38.5%) of the cases showed easy fatigubility as the commonest presenting symptom in megaloblastic anemia followed by fever and generalised weakness, and pallor was the most commonest sign observed seen in 32(45.7%) of the cases followed by splenomegaly and hepatomegaly.

Patients with mixed nutritional anemia presented with fever, generalised weakness and bleeding tendencies. Pallor was the most common sign seen in 30 (42.8%) of the cases among this study group attributable to low hemoglobin levels.

In the study of pancytopenia cases by Kumar et al., hemoglobin ranged from 2.4-7 gm/dl, TLC ranged from 700-3600 cells/mm and platelet count ranged from 1,00,000-1,30,000 cells/mm. In the study done by Jha et al, hemoglobin ranged from 2.3-9.8 gm/dl, TLC ranged from 1200-3900 cells/mm3 and platelet count ranged from 2000-1,37,000 cells/mm. In a study done by B N Gayathri et al in observed that the commonest sign seen in 32(45.7%) of the cases followed by splenomegaly and hepatomegaly.

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In the present study, hemoglobin ranged from 3.1-5 gm/dl; which has an insignificant statistical association (p=0.285), TLC ranged from 3100-4000 cells/cumm; which had a statistically significant association (p=0.032) and platelet count ranged from 76,000-1, 00,000 cells/cumm; which had a statistically insignificant association (p=0.556).
Conflict of Interest: None.

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