Research Article

Etiological Analysis of Pancytopenia in a Tertiary Care Hospital

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Abstract

Background: Pancytopenia refers to the reduction in all the three cell lines of blood; white blood cells, red blood cells and platelets. Its etiology varies with geographical location, genetic predisposition, age and gender.

Objective: To determine the most common etiologies of pancytopenia in a hospital of Lahore.

Methods: It was a cross sectional study conducted at Shalamar hospital after taking approval from the Institutional Review Board of Shalamar Medical and Dental College, Lahore. A purposive sample of one hundred admitted patients fulfilling the inclusion criteria for pancytopenia was taken. History was taken and detailed physical examination was done after taking informed consent. Peripheral smear examination, reticulocyte count, Liver function tests (LFTs), Renal function tests (RFTs), vitamin B12 and folic acid levels and viral serology were carried out along with additional investigations as indicated. For statistical analysis SPSS version 20 was used.

Results: A total of 100 patients, 9 to 82 years of age with pancytopenia were studied. Mean age of studied population was 50.42 ± 2.61 years, there were 52 female and 48 male patients and with a female to male ratio of 1:0.9. The most common cause of pancytopenia was Chronic Liver Disease (CLD) observed in 66%, followed by megaloblastic anemia in 14% and sepsis in 10% of studied patients.

Conclusion: CLD, megaloblastic anemia and sepsis were found to be the most frequent etiologies of pancytopenia in current study.

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Key Words: Pancytopenia, chronic liver disease, megaloblastic anemia, sepsis.

Introduction

Pancytopenia refers to reduction in all the three cell lines of blood i.e. white blood cells, red blood cells and platelets.¹ It is not a disease itself but a manifestation of a number of disease processes affecting the bone marrow. The various mechanisms leading to pancytopenia include failure of bone marrow, unproductive hematopoiesis, space occupying lesions of bone marrow or destruction of hematopoietic cells in the periphery.²,³ Patients may present with features of anemia like pallor, fatigue and shortness of breath, infections due to leucopenia or bleeding due to thrombocytopenia.

The etiology of pancytopenia varies according to geographical location, genetic predisposition, age and gender.² As the management and prognosis rests
on the underlying cause of pancytopenia, identification of the underlying etiology is of utmost importance. A detailed history should be taken including the duration of symptoms, dietary history, exposure to potentially toxic chemicals or drugs, any blood transfusions and occupational exposure. Investigations needed to establish the cause include peripheral smear and reticulocyte count, Liver function tests (LFTs), viral serology, autoimmune profile, folic acid and vitamin B12 levels, examination of bone marrow, cytogenetic analysis, immunophenotyping and abdominal ultrasound.1

A number of studies can be found in the literature regarding the etiology and clinicohematological features of pancytopenia.4,5 Since the underlying causes vary according to age, gender, socioeconomic background of the study population, geographic area, the type of health care facility where the study is performed and its catchment area, the current study was done to determine the most common etiologies of pancytopenia in a tertiary care hospital of Lahore. It will apprise the clinicians of prevalent causes in our set up and help to outline the necessary work up of pancytopenia.

Methods

It was a cross sectional study conducted at tertiary care hospital after taking approval from the Institutional Review Board of Shalamar Medical & Dental College Lahore. A purposive sampling technique was used, a total of one hundred patients admitted in hospital fulfilling the inclusion criteria for pancytopenia were included after taking informed consent. Data was collected in duration of six months.

The inclusion criteria of present study was: (1) Hemoglobin (Hb) level < 13gm/dl for males, < 12gm/dl for females and < 11.5gm/dl for children (2) Total leukocyte count (TLC) < 4 x 10³/µL, (3) Platelet count <150 x 10³/µL, 6 (4) all age groups and both genders.

While exclusion criteria was: (1) Patients who developed pancytopenia because of chemotherapy/radiotherapy, (2) patients with incomplete medical record (3) Patients with mono or bicytopenia.

History was taken and detailed physical examination was carried out. Pulse, systolic and diastolic blood pressure and body temperature was recorded. All the selected patients were thoroughly investigated to ascertain the cause of pancytopenia. Complete determination of blood counts (CBC) with examination of peripheral smear and reticulocyte count, LFTs, Renal function tests (RFTs) and viral serology were carried out in all the patients while autoimmune profile, folic acid and vitamin B₁₂ levels, examination of bone marrow, cytogenetic analysis, immunophenotyping and abdominal ultrasound were performed where indicated by the clinical features and findings of baseline investigations.

CBC was performed on Sysmex XS 500i automated hematology analyzer. For peripheral blood smear examination; slides were stained with Giemsa stain. For reticulocyte count supravital staining was done using New Methylene blue dye and the slides were examined by a hematologist. LFTs and RFTs were carried out on Roche Cobas C311 chemistry analyzer. Viral serology (for hepatitis B and C), autoimmune profile, serum vitamin B12 and folate levels were done by chemiluminescence immunoassay. Aspiration of bone marrow and trephine biopsy was done under local anaesthesia from posterior iliac crest. Smears from bone marrow aspirate were stained with Giemsa and Perl stains. Trephine biopsy was processed according to standard protocol on an automated tissue processor followed by haematoxylin and eosin staining. Findings of abdominal ultrasound (if done) were noted from the files of patients. All data were recorded in a predesigned proforma.

SPSS version 20 was used for statistical analysis, mean ± standard error of mean, range and percentages were calculated. Independent sample ‘t’ test was used to determine the differences between two groups, p value of less than 0.05 was considered significant statistically.

Results

A total of 100 patients were identified as having pancytopenia with a mean age of 50.42 ± 2.61 years. There were 52 female and 48 male subjects, with female to male ratio of 1:0.9. Baseline characteristics of study population are given in Table 1.
Table 1: Baseline characteristics of study population

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total (n=100)</th>
<th>Males (n=48)</th>
<th>Females (n=52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>50.42 ± 2.61</td>
<td>45.71 ± 3.91</td>
<td>54.77 ± 3.33</td>
</tr>
<tr>
<td>Age range (years)</td>
<td>9-82</td>
<td>11-82</td>
<td>9-76</td>
</tr>
<tr>
<td>Pulse rate /min</td>
<td>87.68 ± 1.59</td>
<td>86.42 ± 2.17</td>
<td>88.85 ± 2.33</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>126.4 ± 3.29</td>
<td>123.75 ± 4.81</td>
<td>128.85 ± 4.55</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>76.08 ± 1.44</td>
<td>76.42 ± 2.09</td>
<td>75.77 ± 2.01</td>
</tr>
<tr>
<td>Body temperature (°F)</td>
<td>99.05 ± 0.25</td>
<td>99.37 ± 0.28</td>
<td>98.75 ± 0.41</td>
</tr>
</tbody>
</table>

The highest number (30) of patients with pancytopenia were observed in the age group of 61-70 years, consisting of 10 males and 20 females whereas twenty patients each were found in age groups of 41-50 and 61-60 years. The lowest number of studied patients belonged to age group of 81-90 years in which there were only 02 male patients.

Female patients were more anemic as compared to males as shown by significantly ($p < 0.05$) less hemoglobin concentration when compared to males. Red blood cells, platelets and white blood cells were also less in females compared to males when compared by applying independent sample ‘t’ test, $p$ value of less than 0.05 was considered significant statistically (Table 2).

Table 2: Hematological findings of the study population

<table>
<thead>
<tr>
<th>Hematological Parameters</th>
<th>Total (mean ± SEM)</th>
<th>Males (mean ± SEM)</th>
<th>Females (mean ± SEM)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin Concentration (gm/dL)</td>
<td>8.01 ± 0.27</td>
<td>8.57 ± 0.44</td>
<td>7.48 ± 0.31*</td>
<td>0.043*</td>
</tr>
<tr>
<td>Red Blood Cell Count (10⁶/µL)</td>
<td>2.74 ± 0.09</td>
<td>2.84 ± 0.13</td>
<td>2.65 ±0.14</td>
<td>0.324</td>
</tr>
<tr>
<td>White Blood Cell Count (10³/µL)</td>
<td>2.67 ± 0.21</td>
<td>2.81 ± 0.33</td>
<td>2.55±0.27</td>
<td>0.541</td>
</tr>
<tr>
<td>Platelet Count (10³/µL)</td>
<td>47.68 ± 3.65</td>
<td>49.36 ± 5.50</td>
<td>46.13 ± 4.93</td>
<td>0.662</td>
</tr>
<tr>
<td>Mean Corpuscular Volume (fL)</td>
<td>87.10 ± 1.63</td>
<td>87.87 ± 2.58</td>
<td>86.39 ± 2.07</td>
<td>0.653</td>
</tr>
</tbody>
</table>

Figure 1: Etiologies of pancytopenia in study population

Figure 2: Male to female ratio in each etiology of pancytopenia
In present study Chronic Liver Disease (CLD) was observed to be the commonest cause of pancytopenia, as 66 patients were diagnosed with CLD with 34 females and 32 males. Among all CLD cases, 14 were further diagnosed with Decompensated Chronic Liver Disease (DCLD). The second common etiology associated with pancytopenia was megaloblastic anemia with 14 patients with male to female ratio 2:1. Ten patients had sepsis and in these patients female to male ratio was 1:0.6 (Figure 1 and 2).

Table 3: Laboratory findings of the study population to ascertain the cause of pancytopenia

<table>
<thead>
<tr>
<th>Etiology</th>
<th>Renal Function Test (RFT)</th>
<th>Liver Function Test (LFT)</th>
<th>Viral Serology for Hepatitis B</th>
<th>Viral Serology for Hepatitis C</th>
<th>Autoimmune Profile (ENA Profile)</th>
<th>Serum Vitamin B12 Level (pg/ml)</th>
<th>Serum Folate Level (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum creatinine (mg/dl)</td>
<td>Serum Alanine Aminotransferase (ALT) U/L</td>
<td>Serum Aspartate Aminotransferase (AST) U/L</td>
<td>Reactive/Non-Reactive</td>
<td>Reactive/Non-Reactive</td>
<td>Reactive/Non-Reactive</td>
<td>(mean ± SEM)</td>
</tr>
<tr>
<td>Chronic Liver Disease (CLD)</td>
<td>1.4±0.01</td>
<td>70±0.53</td>
<td>85±0.43</td>
<td>Reactive in 02 cases</td>
<td>Reactive in 52 cases</td>
<td>Non-reactive</td>
<td>N/A</td>
</tr>
<tr>
<td>Megaloblastic Anemia</td>
<td>1.2± 0.05</td>
<td>38±0.06</td>
<td>40±0.05</td>
<td>Non-reactive</td>
<td>Non-reactive</td>
<td>ANA Positive in 03 cases</td>
<td>159±1.11</td>
</tr>
<tr>
<td>Sepsis</td>
<td>1.8±0.1</td>
<td>80±0.09</td>
<td>45±0.1</td>
<td>Non-reactive</td>
<td>Non-reactive</td>
<td>Non-reactive</td>
<td>N/A</td>
</tr>
<tr>
<td>Malaria</td>
<td>1.2±0.2</td>
<td>40±0.3</td>
<td>38±0.4</td>
<td>Non-reactive</td>
<td>Non-reactive</td>
<td>Non-reactive</td>
<td>N/A</td>
</tr>
<tr>
<td>Non-Hodgkin’s Lymphoma</td>
<td>1.5±0.01</td>
<td>38±0.01</td>
<td>36±0.01</td>
<td>Non-reactive</td>
<td>Non-reactive</td>
<td>Non-reactive</td>
<td>N/A</td>
</tr>
<tr>
<td>Celiac Disease</td>
<td>0.9±0.09</td>
<td>58±0.01</td>
<td>40±0.04</td>
<td>Non-reactive</td>
<td>Non-reactive</td>
<td>ANA -positive , anti Ttg- positive</td>
<td>N/A</td>
</tr>
<tr>
<td>Acute Leukemia</td>
<td>1.2±0.2</td>
<td>38±0.05</td>
<td>36±0.06</td>
<td>Non-reactive</td>
<td>Non-reactive</td>
<td>Non-reactive</td>
<td>N/A</td>
</tr>
<tr>
<td>Aplastic Anemia</td>
<td>1.3± 0.09</td>
<td>45±0.03</td>
<td>38±0.1</td>
<td>Non-reactive</td>
<td>Non-reactive</td>
<td>Non-reactive</td>
<td>N/A</td>
</tr>
<tr>
<td>Sjogren’s Syndrome</td>
<td>0.8± 0.1</td>
<td>40±0.12</td>
<td>36±0.11</td>
<td>Non-reactive</td>
<td>Reactive</td>
<td>ANA- positive Anti Ro, Anti La- positive</td>
<td>N/A</td>
</tr>
<tr>
<td>Myelodysplastic Syndrome</td>
<td>1.0±0.1</td>
<td>50±0.17</td>
<td>42±0.15</td>
<td>Non-reactive</td>
<td>Non-reactive</td>
<td>Non-reactive</td>
<td>N/A</td>
</tr>
</tbody>
</table>

It was observed that 79% of CLD patients were Hepatitis C positive whereas only 3% of CLD patients had Hepatitis B. Among all the patients of megaloblastic anemia, 64% exhibited vitamin B12 deficiency with an average vitamin B12 level of 159 pg/ml (Normal range: 200-900 pg/ml) while 14% showed folic acid deficiency with mean folate level of 1.61 ng/ml (Normal range: 2-20 ng/ml in adults), 21% of these patients had mixed deficiency of vitamin B12 and Folic acid (Table 3).
Table 4: Findings of bone marrow aspirate and trephine biopsy

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Bone marrow aspirate</th>
<th>Bone marrow trephine biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non Hodgkins lymphoma</td>
<td>Hemodiluted smears. The only cells seen were small to medium sized lymphoid cells with indented nuclei having homogeneous chromatin. These cells had abundant pale blue cytoplasm with circumferential hairy projections.</td>
<td>Adequate length trephine biopsy with overall cellularity of about 98%. Marrow showed complete effacement by lymphoid infiltrate showing widely spaced cells with oval nuclei and abundant cytoplasm. Areas of increased fibrosis noted.</td>
</tr>
<tr>
<td>a- Hairy cell leukemia</td>
<td>Good cellularity fragments and smears. Trilineage hematopoiesis was present.</td>
<td>Adequate length trephine biopsy with overall cellularity of about 70%. About 40% of the cellular area was infiltrated by small to medium sized lymphocytes having clefted nuclei.</td>
</tr>
<tr>
<td>b- Follicular lymphoma</td>
<td>Adequate length trephine biopsy with increased fat spaces and overall cellularity of about 08%.</td>
<td></td>
</tr>
<tr>
<td>Acute leukemia</td>
<td>Hypercellular fragments and cell trails. Blast cells constituted 90% of cells. Blast cells were small to medium in size, had high N/C ratio, round to oval nuclei with clumped chromatin and inconspicuous nucleoli.</td>
<td>Adequate length trephine biopsy with overall cellularity of about 95%. Marrow showed total effacement by relatively uniform population of blasts having round to oval nuclei with dispersed chromatin.</td>
</tr>
<tr>
<td>Aplastic anemia</td>
<td>Hypocellular fragments with somewhat hemodiluted cell trails. Trilineage hematopoiesis was reduced. No abnormal cell found.</td>
<td>Adequate length trephine biopsy with overall cellularity of about 60%. Erythropoiesis was hyperplastic with prominent megaloblasts.</td>
</tr>
<tr>
<td>Myelodysplastic syndrome</td>
<td>Hyperplastic erythropoiesis with dysplastic changes e.g. multinucularity, inter cytoplasmic bridging. Giant myelocytes and metamyelocytes seen. Iron stain showed 22% ringed sideroblasts.</td>
<td>Adequate length trephine biopsy with overall cellularity of about 60%. Erythropoiesis was hyperplastic with prominent megaloblasts.</td>
</tr>
</tbody>
</table>

Other etiologies in association with pancytopenia included celiac disease (two patients), Non- Hodgkin’s Lymphoma (two patients) and malaria (two patients). Acute leukemia, aplastic anemia, Sjogren’s syndrome and myelodysplastic syndrome were found to be the less common etiologies of pancytopenia. Etiological analysis of pancytopenic patients with Non-Hodgkins lymphoma (including: Hairy cell leukemia and Follicular lymphoma), acute leukemia, aplastic anemia, and myelodysplastic syndrome was based upon their bone marrow aspirate and trephine biopsy findings (Table 4).

Discussion

Pancytopenia is not an uncommon finding in routine clinical practice. Underlying causes vary according to age, gender, geographic area, the type of health care facility and its catchment area and the category of population studied (hospitalized, outpatient).

In present study, mean age of study population was 50.42 ± 2.61 years ranging from 9 to 82 years. This is in accordance with previous studies from Nepal and Mexico where the mean age was reported to be 43.95 years with range of 2-82 years and 49.4 years ranging from 16 to 85 years respectively.7,1 Arshad et al from Pakistan have also reported mean age of their pancytopenic patients as 46.6 years with a range of 6 months to 90 years.8

Regarding gender predilection, previous studies have reported male preponderance for pancytopenia. Male to female ratio was reported as 2.69:1 and 2.5:1 by Hayat et al9 and Arshad et al8 respectively. It may be related to more exposure of males to industrial toxins, pesticides or radiation. Current study did not find any significant gender difference in male to female ratio.
In the present study CLD was observed to be the most important cause of pancytopenia. A previous study from Istanbul also reported CLD to be the major cause of pancytopenia in patients older than 65 years of age. However, they observed CLD in 24% patients which is much lower than our findings (66%). Ishiq et al\textsuperscript{10} in 2004 reported CLD as a cause of pancytopenia in 19% patients. Most likely explanation of this difference is a continuously rising incidence of chronic liver disease in Pakistan. A recent study from Pakistan showed liver diseases to be responsible for more than 1/4\textsuperscript{th} of hospital admissions and 68% of them having decompensated liver disease.\textsuperscript{11} CLD is an important global community health problem accounting for about two million deaths annually.\textsuperscript{12} In Pakistan CLD is the eleventh most frequently observed cause of disability and fifth among the commonest causes of death.\textsuperscript{13} Pancytopenia in such patients is related to bone marrow suppression, hypersplenism and antiviral therapy.

Hepatitis B and C, diabetes mellitus, obesity, non-alcoholic steatohepatitis (NASH), use of herbal and food supplements are common risk factors of CLD in Pakistan\textsuperscript{12}. In present study, 79% cases of CLD were Hepatitis C positive. Our findings are consistent with those of Butt et al who have documented Hepatitis C as the common (55.8%) cause of chronic liver disease.\textsuperscript{14}

In our study, the prevalence of Hepatitis C was found to be higher than that of Hepatitis B as the cause of pancytopenia. Butt et al\textsuperscript{14} have also reported increasing tendency in HCV and falling trend of hepatitis B in Pakistan. It could be attributable to the increase in efforts for HBV vaccination and awareness and early detection of hepatitis C in Pakistan.

Second important cause of pancytopenia was megaloblastic anemia and it was observed in 14% of the cases. Arshad et al\textsuperscript{8} have reported megaloblastic anemia in 25.7% of their patients and it was observed to be the major cause of pancytopenia. The results of the present study are in agreement with those of Hayat et al\textsuperscript{9} who observed that in patients with pancytopenia, megaloblastic anemia was present in 17.6%. In megaloblastic anemia there is failure of nuclear maturation due to diminished DNA synthesis. The megaloblastic anemia is due to deficiency of vitamin B\textsubscript{12} or folic acid. A large number of cells with defective formation experience programmed cell death (ineffective hematopoiesis) leading to cytopenias. Megaloblastic anemia may be due to dietary deficiency of cobalamin and folic acid, poor absorption of these vitamins by the intestines or may be due to inappropriate consumption of these vitamins by the body. Folic acid deficiency may also result from the conditions in which there is increase in utilization or requirement of folate. In present study, among all patients of megaloblastic anemia, 64% exhibited Vitamin B\textsubscript{12} deficiency, 14% showed Folic acid deficiency while 21% had mixed deficiency. Our results are consistent with those of Huang et al\textsuperscript{15} who reported vitamin B\textsubscript{12} deficiency in 78\% of patients with megaloblastic anemia and folate deficiency in only 4\% cases while 18\% were having combined deficiency. Jan et al\textsuperscript{16} have also reported vitamin B\textsubscript{12} deficiency to be more common cause of megaloblastic anemia than folate deficiency.

In current study, sepsis was found in 10 patients. Jain et al\textsuperscript{17} have reported sepsis to be a cause of pancytopenia in 5.6\% of their patients. Sepsis is considered as a major cause of pancytopenia, leading to cytopenias through a combination of mechanisms (marrow suppression, hypersplenism, consumptive coagulopathy). Sepsis induced marrow suppression due to hematopoietic stem cell dysfunction is well documented. Zhang et al\textsuperscript{18} have reported that downstream signaling of Toll-like receptor 4 (TLR4) is responsible for suppression of bone marrow and exhaustion of hematopoietic stem cell during sepsis. Bone marrow depression can also be a side effect of drugs used to treat infections.\textsuperscript{19} This effect may be dose dependent or idiosyncratic. Early recognition and withdrawal of offending drug usually resolves pancytopenia. Severe and persistent marrow suppression may require treatment with intensive immunosuppression or consideration of bone marrow transplantation.

Widespread activation of coagulation due to disseminated intravascular coagulation (DIC) can occur in severe sepsis. Activation of diffuse coagulation occ-
urs by cell-specific components in the membrane of the microorganism, such as lipopolysaccharide, bacterial exotoxins or endotoxin.\(^{20}\)

Another phenomenon leading to pancytopenia is acquired hemophagocytic lymphohistiocytosis that should not be ignored in sepsis\(^ {21}\). It results from the deregulated activation and proliferation of lymphocytes, leading to overproduction of cytokines.

Other etiologies in association with pancytopenia included celiac disease, NHL and Malaria having two patients each in the present study. Celiac disease is associated with many hematological manifestations mostly due to failure of absorption of iron, vitamin B\(_{12}\) and/or folic acid. However, the occurrence of aplastic anemia with celiac disease has also been reported.\(^ {22}\) In both diseases underlying immune mechanism is the likely explanation for association.

Out of two cases of NHL, one patient had Hairy cell leukemia and the other, follicular lymphoma. Pancytopenia was the result of extensive marrow infiltration. Diagnosis was confirmed on bone marrow examination and immunophenotyping.

Pancytopenia can occur in malaria due to splenomegaly, hemolysis, necrosis of bone marrow or suppression of marrow function.\(^ {23}\) While Hamid et al\(^ {24}\) reported malaria in 17.3% of their pancytopenic patients, we found malaria in only two patients. Malaria is usually associated with thrombocytopenia alone or in combination with anemia. It causes pancytopenia in severe cases. As we excluded the patients having mono or bicytopenia from our study, we may have missed cases of malaria.

Although acute leukemia\(^ {1}\), aplastic anemia\(^ {9}\), autoimmune disorders\(^ {25}\) and myelodysplastic syndromes\(^ {2}\) are reported to be notable causes of pancytopenia in previous studies, these were found to be the uncommon causes of pancytopenia in the present study. This supports the fact that leading causes of pancytopenia vary according to geographical location, age, gender, genetic predisposition and socio economic status of the studied population. The evaluation of a patient with pancytopenia requires a comprehensive approach; identification of the underlying cause is of utmost importance for proper management. The clinicians should be aware of the different causes of pancytopenia present in their local set up so that delay in diagnosis can be prevented along with unnecessary investigations.

**Limitations**

The study focused on admitted patients of only one hospital for evaluation of etiology of pancytopenia. To address this limitation, it is recommended to conduct a study with a larger sample size, including data of indoor as well as outdoor patients from different hospitals of Lahore, Pakistan.

**Conclusion**

Chronic liver disease was found to be the most common etiology followed by megaloblastic anemia and sepsis in the current study.

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**Conflict of Interest**

Authors declare no conflict of interest.

**References**


