Estimation of Thrombocytopenia in Patients of COVID-19 in a Tertiary Care Centre as a Prognostic marker

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Abstract

Introduction: Severe COVID-19 can lead to critical illness, with Acute Respiratory Distress (ARDS) and Multi-organ Failure (MOF) as its primary complications, eventually followed by intravascular coagulopathy. Haematological changes are common in patients with COVID-19, which include reduced lymphocyte count and platelet count but normal white blood cell count and prolonged activated partial thromboplastin time. Using a simple test like platelet count for assessing the risk of mortality and early identification of severe cases will help in preventing the life threatening complications in patients of COVID-19. Aims and Objectives: To study trends of thrombocytopenia in patients of COVID-19 and to study the correlation between thrombocytopenia and severity of cases of COVID-19. Materials and Methods: The study was carried out in Central Clinical Laboratory in a tertiary care centre. A total of 138 random subjects who were admitted in the COVID ICU were included after they satisfied the eligibility criteria. The CBCs were analyzed on the Beckmann Coulter automated cell count analyzer with EDTA samples obtained from peripheral venipuncture of the patients. Platelet trends over the three samples were studied. Results: An average of all three platelets counts for the patients revealed an overall decreasing trend in cases of non survivors, whereas an overall upward trend was noted in the survivors. A total of 79 patients showed thrombocytopenia (platelet count less than 1.5 lakhs/mm³), during at least one of the tests.46 (33.33%) of these patients succumbed, whereas 33 (23.9%) patients survived. Decreasing trends or overall decreasing trends (Increasing then decreasing) were observed in larger number of non survivors as compared to survivors. Also increasing or overall increasing trends (decreasing then increasing) were common in the survivors. Discussion: Hematological changes are common in SARS patients. For thrombocytopenia, the possible mechanisms of SARS-CoV associated thrombocytopenia may include, 1. Direct infection of megakaryocytes and platelets potentially, inducing cell apoptosis and growth inhibition and/or 2. Immune damage of megakaryocyte progenitor cells or platelets; In addition, the lung damage in SARS patients may also play a role in inducing thrombocytopenia. Conclusion: In this study, we found that platelet count may be a simple, economic, rapid and commonly available laboratory parameter that could straightforwardly discriminate between COVID patients with and without severe disease, while the study of serial platelets counts as trends could help identifying those with a serious risk of mortality.

Keywords: COVID-19, Platelet Trends, Prognostic Marker; Thrombocytopenia

1. Introduction

Since December 2019, many patients with Coronavirus disease 2019 (COVID-19) pneumonia have been discovered in Wuhan, Hubei province, China. This virus subsequently spread to other provinces in China and patients have been discovered in other countries¹-³. Novel Coronavirus pneumonia is a novel respiratory disease
in humans that is caused by the novel Coronavirus. The WHO has officially named this disease Coronavirus disease 2019 (COVID-19). Currently, six Coronaviruses that can infect humans have been discovered (HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV and MERS-CoV). The first four viruses mainly cause the common cold, whereas the SARS-CoV and MERS-CoV viruses cause severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), respectively. The newly discovered Coronavirus is a β-coronavirus that has enveloped virus particles that are spherical or oval in shape. Although it belongs to the same genus as SARS-CoV and MERS-CoV, its genetic characteristics show significant differences compared with SARS-CoV and MERS-CoV. After assessment of the virus, the Coronavirus Study Group of the International Committee on Virus Taxonomy recommended naming this virus severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2). The epidemiological data provided by Huang et al. showed that the Huanan Seafood Wholesale Market in Wuhan was the source of the zoonosis. The appearance of disease clusters proved that human-to-human transmission is present. Some researchers found that the full length genome sequence of SARS-CoV-2 obtained from earlier patients had a homology of 79.5% with the SARS-CoV sequence and a homology of 96% with the whole genome of bat Coronaviruses. This provided valuable clues for examining the pathogenesis and clinical treatment of COVID-19.

COVID-19, caused by the severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2), produces a respiratory and systemic illness which progresses to a severe form of pneumonia in 10-15% of patients. Severe COVID-19 can lead to critical illness, with Acute Respiratory Distress (ARDS) and Multi-organ Failure (MOF) as its primary complications, eventually followed by intravascular coagulopathy. The most common symptoms seen in COVID-19 patients are fever, fatigue and dry cough and dyspnoea gradually develops. Some patients have mild symptoms at disease onset and may not present with apparent fever. Uncommon symptoms include abdominal pain, headache, palpitations and chest pain. Haematological changes are common in patients with COVID-19, which include reduced lymphocyte count and platelet count but normal white blood cell count and prolonged activated partial thromboplastin time.

Uniquely to COVID-19, a wide range of variability in disease severity is observed ranging from asymptomatic to critical. As such, biomarkers are needed to identify severe disease among hospitalized patients. In this study, the objectives were to use the platelet count which is a simple, economic, rapid and commonly available laboratory parameter that could potentially discriminate between COVID patients with and without severe disease.

Thrombocytopenia is commonplace in critically ill patients and usually suggests serious organ malfunction or physiologic decompensation as opposed to primary hematologic etiology, as well as the development of intravascular coagulopathy, often evolving towards Disseminated Intravascular Coagulation (DIC).

In COVID-19 patients, the mechanism for thrombocytopenia patients is likely multifactorial. In SARS, it was suggested that the combination of viral infection and mechanical ventilation leads to endothelial damage triggering platelet activation, aggregation and thrombosis in the lung, causing vast platelet consumption. Moreover, as lung may be a site of platelet release from fully mature megakaryocytes, a decrease or morphologic alteration in the pulmonary capillary bed may lead to deranged platelet defragmentation.

Coronaviruses may also directly infect bone marrow elements resulting in abnormal hematopoiesis or trigger an auto-immune response against blood cells. It has also been suggested that a consistently present low grade DIC may propagate a low platelet count in SARS. However, as noted by the World Health Organization (WHO), significant differences are observed between SARS and COVID-19. As such, the pathophysiologic mechanisms behind each infection are likely to differ.

In this study, we studied the trends of platelet counts in patients with severe COVID-19 that is those who needed ICU admission.

2. Need for Study

With the increasing number of cases of COVID-19 along with an increase in the number of asymptomatic cases, it is necessary to identify and utilise the available resources to the optimum. With the grim situation of COVID-19 all around the world, it becomes essential to have early identification of severe cases and thereby, help in prevention of complications from the disease. In order to optimize patient care and resource allocation...
during this pandemic, biomarkers are urgently needed for stratifying patients' risk and for actively monitoring illness severity. Platelet count is a simple and readily available biomarker, which is independently associated with disease severity and risk of mortality in the Intensive Care Unit (ICU)\textsuperscript{13–15}. Moreover, a low platelet count correlates with higher disease severity scores such as Multiple Organ Dysfunction Score (MODS), Simplified Acute Physiology Score (SAPS) II and Acute Physiology and Chronic Health Evaluation (APACHE) II\textsuperscript{14}. In the Severe Acute Respiratory Syndrome (SARS) outbreak, thrombocytopenia was reported to occur in up to 55% of patients and was identified as a significant risk factor for mortality\textsuperscript{7,16}. Platelet count, with hypoxemia, were the only two variables used by Zou \textit{et al}. for developing a SARS prognostic model which displayed 96.2% accuracy\textsuperscript{17}.

In the present study, we aim to investigate whether platelet count could differentiate between COVID-19 patients with or without severe disease and assess if thrombocytopenia may be associated with severe COVID-19.

Using a simple test like platelet count for assessing the risk of mortality and early identification of severe cases will help in preventing the life threatening complications.

3. Aims and Objectives

• To study trends of thrombocytopenia in patients of COVID-19.

• To study the correlation between thrombocytopenia and severity of cases of COVID-19.

• To study the correlation between thrombocytopenia and mortality of cases of COVID-19.

4. Materials and Methods

The study was carried out in Central Clinical Laboratory in a tertiary care centre. A total of 138 random subjects who were admitted in the COVID ICU were included after they satisfied the eligibility criteria. The first blood sample was collected from the patients within 24 hours of their admission to the hospital, before the administration of antibiotics. Follow up samples were collected from the patients on the 3\textsuperscript{rd} day (1\textsuperscript{st} follow-up) and the 6\textsuperscript{th} day (2\textsuperscript{nd} follow-up) following the admission. All privacy and confidentiality safeguards were observed.

CBCs were performed on the EDTA samples collected from the patient.

The CBCs were analyzed on the Beckmann Coulter automated cell count analyzer with EDTA samples obtained from peripheral venipuncture of the patients. Platelet trends over the three samples were studied.

In case a patient was discharged or succumbed before all three tests, the trends were based on the two counts. In case, the patient had just one platelet count and was discharged or succumbed before the first follow up test, that patient was excluded from the study, as studying platelet trend was not possible.

• Inclusion Criteria For Patients:
  • Patients who were diagnosed as positive for SARS-COV-2 by RNA. Detection on the RT-PCR viral RNA detection.

• Exclusion Criteria For Patients:
  • Patients who are known/Diagnosed cases of ITP.
  • Patients already on warfarin/heparin/anti-coagulant treatment.
  • Patients diagnosed with other viral hemorrhagic fevers (eg: dengue).
  • Patients who are diagnosed cases of haematological malignancies.

5. Results

A total of 138 patients which were admitted in the COVID ICU (those who had saturation levels less than 94% on room air) were included in the study. The outcome was decided on the basis whether the patient was discharged or succumbed to the disease.

Out of the 138 patients, 99 were male (71.7%) and 39 were female (28.3%). The average age was 55.6 years, range being 18-85 years. There were 50 patients (36.24 %) above the age group of 60 years, 69 patients (50%) in the age group of 40-60 years and 19 patients (13.76%) whose ages were less than 40 years.

The study had 74 patients (53.7 %) patients who were discharged and 64 (46.3%) patients who succumbed either due to COVID-19 or due to its complications. Out of the 64 patients who succumbed, 38 patients (59.38%) were in the age group of above 60 years, 22 patients (34.37%) were in the age group 40-60 years and 4 patients (6.25%) were less than 40 years of age.
There were a total of 9 patients for whom just two counts of platelet (one on the day of admission and the other one on the 3\textsuperscript{rd} day of admission) could be obtained. Out of these 9, 7 patients succumbed to the disease before their 2\textsuperscript{nd} follow-up (on the 6\textsuperscript{th} day of admission), whereas the other 2 patients, recovered and were discharged. The platelet studies revealed 4 trends:

- Increasing trend (Platelet count on admission < Platelet count of first follow-up < platelet count of second follow-up).
- Decreasing trend (Platelet count on admission > Platelet count of first follow-up > platelet count of second follow-up).
- Increasing then decreasing (Platelet count on admission < Platelet count of first follow-up > platelet count of second follow-up).
- Decreasing then increasing (Platelet count on admission > Platelet count of first follow-up < platelet count of second follow-up).

The following (Table 1) reveals the number of patients in each of the trends:

An average of all three platelets counts for the patients revealed an overall decreasing trend in cases of non-survivors, whereas an overall upward trend was noted in the survivors (Table 2).

A total of 79 patients showed thrombocytopenia (platelet count less than 1.5 lakhs/mm\(^3\)), during at least one of the tests. 46 (33.33\%) of these patients succumbed, whereas 33 (23.9\%) patients survived (Table 3).

Our studies show correlations with various other studies done to study the usefulness of presence of thrombocytopenia as a predictive marker for the prognosis and severity of the disease.

In a study by Choi, \textit{et al}.\(^{18}\), 50\% of the patients suffered from thrombocytopenia, whereas a study by Wong, \textit{et al}.\(^{19}\), shows 55\% of the patients suffering from thrombocytopenia. Similar results were also obtained by Lee, \textit{et al}.\(^{20}\), with 44.8\% patients, Vu \textit{et al}.\(^{21}\), with 40.3\% patients, Liu, \textit{et al}.\(^{22}\) with 40\% patients and Peiris, \textit{et al}.\(^{23}\) with 40\% patients.

The association of thrombocytopenia and other viral infections has been described before and could be immune in origin or due to the direct effects of virus on megakaryocytes and platelets or due to lung damage caused by the virus itself. According to the best of our knowledge, ours is the first study comparing the trends of thrombocytopenia in patients of COVID-19.

Study of trends of platelet count revealed an interesting finding. Decreasing trends or overall decreasing trends

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Study of platelet trends in patients of COVID-19</th>
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<tbody>
<tr>
<td>Outcome of the patient</td>
<td>Platelet trends</td>
</tr>
<tr>
<td>Succumbed</td>
<td>09 (14.07%)</td>
</tr>
<tr>
<td>Discharged</td>
<td>25 (33.78%)</td>
</tr>
<tr>
<td>Total</td>
<td>34 (24.7%)</td>
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<th>Table 2</th>
<th>Average platelet counts</th>
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<tbody>
<tr>
<td>Average platelet counts in lakhs/mm(^3)</td>
<td>Outcome of the patients</td>
</tr>
<tr>
<td>Platelet counts on admission (P1)</td>
<td>1.94</td>
</tr>
<tr>
<td>Platelet counts during first follow-up (3\textsuperscript{rd} day) (P2)</td>
<td>1.85</td>
</tr>
<tr>
<td>Platelet counts during second follow-up (6\textsuperscript{th} day) (P3)</td>
<td>1.61</td>
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<tr>
<th>Table 3</th>
<th>Distribution of patients, according to time when thrombocytopenia was seen</th>
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<tbody>
<tr>
<td>Thrombocytopenia seen in</td>
<td>Succumbed</td>
</tr>
<tr>
<td>P1 only</td>
<td>04</td>
</tr>
<tr>
<td>P2 only</td>
<td>03</td>
</tr>
<tr>
<td>P3 only</td>
<td>10</td>
</tr>
<tr>
<td>P1 and P2</td>
<td>02</td>
</tr>
<tr>
<td>P2 and P3</td>
<td>04</td>
</tr>
<tr>
<td>P1 and P3</td>
<td>12</td>
</tr>
<tr>
<td>P1,P2,P3 (All 3 counts)</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
</tr>
</tbody>
</table>
(Increasing then decreasing) were observed in larger number of non survivors as compared to survivors. Also increasing or overall increasing trends (decreasing then increasing) were common in the survivors.

Our study has several limitations. First, it was retrospective and relied on data collected from case records. Therefore, we may have missed important information in some patients. Our study was limited by variable definition of disease severity among the studies, bias of which, we in part, mitigated through subgroup analysis. Moreover, different cut-offs for thrombocytopenia limits interpretations of that analysis. High heterogeneity suggests inherent variability in platelet levels among patients.

6. Discussion

In the presence of this rapidly emerging, novel infection uncharacteristic of the era of modern medicine, identification of biomarkers that could predict disease severity and prognosis are essential to guiding clinical care. The situation of COVID-19 is very grim worldwide and with increasing number of cases and deaths related to COVID-19 it has become very crucial to identify the high risk patients. Platelet count is a simple and readily available biomarker, which is independently associated with disease severity and risk of mortality in the Intensive Care Unit (ICU). Using a simple test like platelet count for assessing the risk of mortality and early identification of severe cases will help in preventing the life threatening complications.

The possible mechanism of thrombocytopenia in COVID-19:

- SARS-CoV-2 may reduce platelet production. Coronaviruses are able to infect bone marrow cells, resulting in abnormal hematopoiesis. SARS-CoV-2 and human SARS-CoV have 82% nucleotide homology. Because SARS-CoV and HCoV-229E have identical antigen characteristics, it is speculated that SARS-CoV-2 and HCoV-229E antigens have some similarity. Human aminopeptidase N (CD13) is a metalloprotease that is present on the cell surfaces of epithelial cells in the intestine, kidneys and lungs and is a receptor for HCoV-229E. CD13 is a marker of granulocytes and monocytes and is ubiquitous in respiratory tract epithelial cells, smooth muscle cells, fibroblasts, epithelial cells in the kidneys and small intestine, activated endothelial cells, lymphocytes and platelets. HCoV-229E enters bone marrow cells and platelets through CD13 receptors and induces growth inhibition and apoptosis in the bone marrow, leading to aberrant hematopoiesis and thrombocytopenia. Thrombocytopenia caused by SARS-CoV-2 infection is similar to that caused by SARS-CoV and HCoV-229E infection. Based on this phenomenon, it is speculated that SARS-CoV-2 similarly inhibits hematopoiesis in the bone marrow through certain receptors to cause decreased primary platelet formation and lead to thrombocytopenia. Secondary hemophagocytic lymphohistiocytosis (sHLH) is caused by excessive proliferation and activation of mononuclear macrophage system, in which a large number of inflammatory cytokines are released and a large number of blood cells are swallowed. This reactive disease has a rapid response with high mortality and its basic features include persistent fever, hyperferremia, cytopenia and lung involvement. In the retrospective analysis of 150 patients with COVID-19 in Wuhan, China, it was found that elevated ferritin was one of the predictors of death. After analyzing the blood samples of 33 severe and critical type ill COVID-19 patients, Wei Haiming's team found that after novel Coronavirus infection, T cells were over activated to produce Granulocyte-macrophage Colony-stimulating Factor (GMCSF)
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and interleukin-6 (IL-6). GM-CSF stimulated CD14+ CD16+, inflammatory mononuclear macrophages to produce more interleukin-6 (IL-6), and other inflammatory factors, thus forming an inflammatory storm and causing immune damage to the lungs and other organs27. This is similar to the clinical manifestation and laboratory examination of patients with sHLH. In addition, studies have shown26 that the cytokine spectrum similar to sHLH is related to the severity of COVID-19 disease. It is speculated that after the cytokine storm, the hematopoietic progenitor cells in bone marrow of patients with pneumonia infected by novel Coronavirus were destroyed, the primary production of platelets decreased and at the same time, too many blood cells were swallowed, which led to the decrease of peripheral blood platelet count. Evidence28 has shown that a large number of megakaryocytes dynamically release platelets during pulmonary circulation. Persistent hypertension and oxygen toxicity exacerbate lung injury, resulting in consolidation changes such as fibrosis. Damaged pulmonary capillary beds cause the process of megakaryocyte rupture and platelet release to be blocked, which affects the release of platelets into the pulmonary circulation and indirectly leads to reduced platelet synthesis in the systemic circulation.

- SARS-CoV-2 infection may increase platelet destruction - COVID-19 may increase levels of auto-antibodies and immune complexes, resulting in specific destruction of platelets by the immune system. A study reported that the phenomenon of immune-mediated thrombocytopenia in patients infected with HIV-1 is widespread29. Although the pathogenesis is unknown, this was proven to be associated with circulating immune complexes containing platelet membrane components and the anti-platelet membrane GPIIIa49-66 IgG antibodies30. Anti-platelet membrane GPIIIa49-66 IgG antibodies can cross-react with the HIV-1GP 160/120 antigen. Antibodies and immune complexes deposited on the surfaces of platelets will be recognized by reticuloendothelial cells and the platelets will be destroyed as target tissues, resulting in excessive platelet destruction. Platelets with similar antigens may be coated by anti-platelet antibodies and immune complexes, which may result in immune-mediated damage. In addition, antibodies produced during viral infection may specifically bind to antigens on platelets through molecular mimicry, resulting in increased platelet destruction.
- SARS-CoV-2 infection may increase platelet consumption - Viral infection and inflammation result in lung damage. Damaged lung tissues and pulmonary endothelial cells may activate platelets in the lungs, resulting in aggregation and formation of microthrombi, which increases platelet consumption. Most patients with COVID-19 who have thrombocytopenia have elevated D-dimer levels and impaired coagulation time. Therefore, it is still unclear which drugs used for the treatment of patients with COVID-19 having thrombocytopenia resulted in recovery. SARS-CoV-2, MERS-CoV and SARS-CoV are all β-coronaviruses. Previously, a patient with MERS received large doses of corticosteroids by intravenous infusion to treat thrombocytopenia and their platelet counts improved30. This classical method has been shown to correct thrombocytopenia in patients infected with HIV31. Therefore, it is speculated that the intravenous injection of human immunoglobulin, corticosteroids and platelets may be effective in patients under certain circumstances. Reverse transcriptase inhibitors are effective in the treatment of HIV-related thrombocytopenia. For example, zidovudine increased platelet synthesis. In addition, drug stimulation of megakaryocyte synthesis can increase platelet synthesis. Evidence shows that the chemokine CXCR4 can be expressed in megakaryocytes. Because SARS-CoV-2 and HIV are both RNA viruses, reverse transcriptase inhibitors and chemokine receptor antagonists may improve the disease course of COVID-19. The monoclonal antibody against IL-6 receptor tocilizumab can effectively block COVID-19’s inflammatory storm, thus improving the prognosis of the patients32.

7. Conclusion

Hematological changes are common in SARS patients. For thrombocytopenia, the possible mechanisms of SARS-CoV associated thrombocytopenia may include, 1. Direct infection of megakaryocytes and platelets potentially, inducing cell apoptosis and growth inhibition and/or 2. Immune damage of megakaryocyte progenitor cells or platelets; In addition, the lung damage in SARS patients may also play a role in inducing thrombocytopenia.
In this study, we found that platelet count may be a simple, economic, rapid and commonly available laboratory parameter that could straightforwardly discriminate between COVID patients with and without severe disease, while the study of serial platelets counts as trends could help identifying those with a serious risk of mortality.

8. References


