megakaryocyte. Hence the lymphocytic and platelet levels can be used as an indicator of any kind of infection and inflammation. But still we don't know whether these indicators can be used with similar clinical value for COVID-19.

On analysis of the most probable etiology for platelet changes in COVID-19 patient were due to inhibiton of haematopoeisis due to direct invasion of bone marrow or the haematopoietic stem cell. In SARS-CoV2 infection, megakaryocyte may mature in lungs to increase local platelets and cause thrombocytopenia in blood circulation. Injury to lung parenchyma and endothelial cells can lead to activation and aggregation of platelets in the lungs and thrombus formation in pulmonary vasculature. Finally the decreased blood supply distally may decrease the platelet generation and increase their consumption [3].

Also some studies have shown that the total leucocyte count, absolute neutrophil count, neutrophil to lymphocyte ratio and platelet to lymphocyte ratio were highest in the severely symptomatic group and lowest in the asymptomatic group. Neutrophil to lymphocyte ratio was positively associated with a risk of COVID-19 pneumonia [4]. Severe disease was associated with significant neutrophilia and lymphopenia further intensified in critically ill patients [5].

Abnormal WBC morphology was more pronounced in monocytes and lymphocytes in milder form of disease; the changes were lost with disease progression. Higher RNA content in monocytes, lower RNA content in lymphocytes and smaller hypo-granular neutrophils were found [6].

Objective: To review the platelet to lymphocyte and neutrophil to lymphocyte ratio in COVID-19 positive patient and finding out their prognosis.

Methodology: An extensive search strategy was designed to identify papers on COVID-19 and a systemic literature review was used to carry out using Medline, PubMed, Google scholar, EMBASE databases. We have included articles which focus on complete blood count in COVID-19 patients. Preference is given to recently published articles. Articles chosen are relevant and focus on mainly the COVID-19 second wave. We use guidelines issued from ICMR and WHO on their website for study analysis. Data used are announced by health ministry of India.

LITERATURE REVIEW

The COVID-19 disease is caused by a newly emerged mutant of corona virus that is SARS-CoV-2. The virus is highly contagious and infects through respiratory route. It invades the respiratory tract mainly lungs causing coronavirus pneumonia. Various studies have shown the clinical manifestations among the patients as fever, non-productive cough, breathlessness, myalgia, fatigue. In severe cases, disease can rapidly progress to ARDS (acute respiratory distress syndrome), septic shock, MODS (multi-organ dysfunction syndrome) and in the convalescent period presenting with cough, chest tightness, breathlessness and fatigue, tongue redness or a thick, greasy or white coating on the tongue. Some people have also experienced certain neurological and psychiatric problems, post COVID fatigue syndrome in post recovery phase.

Rapid and accurate diagnosis of COVID-19 is essential to prevent the outbreaks of COVID-19 in an on-going pandemic. Currently, two molecular tests rapid antigen test and reverse transcriptase polymerase chain reaction are being used for the diagnosis. The diagnostic criteria was made by china officials which included nasopharyngeal and oropharyngeal swab tests have become a standard for diagnosis of COVID-19 infection but remains unreliable due to variable turnaround time and a high false-negative rate.

Erythrocyte parameters like lower haemoglobin levels, low levels of haematocrit, and increased Red cell Distribution Width (RDW). White blood cell parameters like lower leukocyte levels. Increased neutrophils, lymphopenia and eosinopenia relatively in patients with severe diseases. Thus, causing elevated NLR and MLR.

Platelet parameters like thrombocytopenia, increased PLR and increased MPV. Lymphocyte count, neutrophil count, platelet count and the respective NLR, MLR and PLR ratio as a prognostic marker have been studied thoroughly. Morphological changes in the inflammatory cells have also been studied.

Erythrocyte parameters

Haemoglobin and RBC count: anaemia in COVID-19 may originate from the cytokine storm mediated suppression of the bone marrow and shortened lifespan of Red blood cells. SARS CoV-2 causes hypoxia by affecting the lungs. Also the virus induces inflammation in the tissues affecting metabolism of iron. The immune response of an SARS CoV-2 infected person also alters iron metabolism. The innate immune response activates the signalling pathways to increase hepcidin hormone thus inhibiting the ferroportin transporter and decreased total iron absorption from the digestive tract. Prolonged infection with the virus leads to iron deficiency. Virus also damages the bone marrow decreasing the erythropoiesis thus causing further decrease in haemoglobin levels. Thrombosis in renal arterial supply may lead to kidney injury and thus decreasing the erythropoietin production from the kidney leading to more severe anaemia [7,8].

RDW: Raw signifies the red cell distribution width that is the anisocytosis or variation in size and volume of the red blood cells. The viral damage to bone marrow causing impaired erythropoiesis leads to liberation of immature RBC's into the circulation, this further cause's compensatory hyperplasia of erythroid cell lineage in bone marrow thus causing further immature cells into circulation. Viral inflammation alters the structural protein needed in erythropoiesis, the micro thrombi formation due to the coagulation abnormalities SARS CoV-2 infection leads to disruption of cell membrane

structural integrity. Decreased erythropoietin production by the kidney also leads to anisocytosis and increases RDW significantly. Increased RDW is found to be associated with severe COVID-19 disease, severe AKI-acute kidney injury and other complications [9-11].

Leucocyte parameters

Leucocyte count: Patient with COVID-19 is found to have lower total no. of leucocytes as compared to normal individuals. But patients with severe disease are found to have much higher no. of leucocytes than the mild to moderate disease [12,13].

Differential leucocyte count: SARS CoV-2 infected patients are found to have lower eosinophil and lymphocyte count and higher neutrophils and monocytes as compared to normal healthy individuals.

Eosinophils: Various studies have shown plenty of 19 patients had either lower eosinophil count or absolute absence of eosinophils. Patients with complete absence of eosinophils showed higher mortality as compared to those with eosinopenia. Thus eosinophil count can be used to assess the probability of developing a severe disease [14]. Patients with early bounce up of eosinophils had a lesser severity over the course of disease as compared to those with delayed bounce up of eosinophils [13]. Eosinopenia may be due to damage to bone marrow. Inflammatory cytokines induced apoptosis could be another factor for eosinopenia. Increased neutrophils and monocytes are found in almost all the bacterial and viral infections.

Neutrophils: Neutrophils have their important role in protection from the infection organism and restricting their proliferation in the body. Patients having severe infection have lowered immune response from the body thus making them more prone to bacterial co-infections leading to a further increase in neutrophil levels [15].

Monocytes: As we know SARS CoV-2 is similar to MERS virus, the pathophysiology involved in monocytosis could be related to CoV-2 also. Viral infection tends to trigger some cytokine mediated pathways like IL-6 and IFN-alpha and chemokine like IL-8, CXCL-10 and CCL-5 lead to increase in monocyte levels. Patients with higher level of monocytes are found to have a severe disease and increased mortality as compared to those with lower levels of monocytes in circulation [12,16].

Lymphocytes: Lymphocytes have a definitive role in the innate immunity of a person. The COVID-19 virus has been shown to cause direct damage to the lymphocytes. The virus has ability to infect the T-lymphocytes by attaching to CD147 spike protein receptor and angiotensin converting enzyme 2 receptor present on the cell membrane of lymphocytes. Thus this becomes a vicious cycle of infecting more and more lymphocytes and proliferating viruses within the lymphocytes and degradation of lymphocytes in this process which leads to lymphopenia in COVID-19 infection. This also leads to a prolonged inflammatory state and unintervened T-cell stimulation leading to T-cell exhaustion. Exhausted lymphocytes are unable to function and are not able to limit the infection [16].

Lymphopenia has been associated with higher ICU admissions. Also higher levels of lactate dehydrogenase are found to have poorer course of disease. The ICU patients were found to have lower CD45+, CD3+, CD4+, CD8+, CD19+ and CD16/56+ counts. Unlike other viral infections like Human immunodeficiency virus, Cytomegalovirus, CD4/CD8 ratio is unaltered in COVID-19 patients. Rather, body immune response is tremendously increased along with release of cytokine storm. Absolute lymphocyte count thus can be considered as a surrogate marker of ineffective immune response against the SARS-CoV-2 infection. Lymphopenia could be due to localisation of lymphocytes at the disease site which have migrated from peripheral circulation. Another reason for lymphopenia could be cytokine storm as previously discussed.

Uncontrolled infection and inflammation lead to cytokine storm which leads to apoptosis of lymphocytes and haunt the lymphocyte production and maturation from bone marrow and lymphoid organs leading to further lymphopenia. Increased lactic acid levels inhibit lymphogenesis. Splenic atrophy and lymph node necrosis has also been reported in some cases [9,17].

NLR and MLR: Increased neutrophils, monocytes and decreased lymphocytes have acquired the spotlight in proportionality to severity of COVID-19 disease. Thus making NLR and MLR an independent risk factor. Various studies have shown that patients with higher NLR have shown to acquire a more severe disease and higher mortality as compared to those with lower NLR [17,18].

Measurement of TLC and DLC is widely available, affordable and efficient and effective manner to identify the patients with risk of developing severe disease and higher mortality.

Platelet parameters

Platelet count and PLR (platelet to lymphocyte ratio: Thrombocytopenia may be due to direct attack of the virus on the bone marrow. Bone marrow has ACE-2 receptors, thus being infected by the virus leading to haematopoetic cellular apoptosis. Liver also has ACE-2 receptors; viral infection of the liver prevents thrombopoetin production from the liver. This prevents thrombopoeisis, platelet differentiation and maturation in bone marrow. Several antibodies and immune complexes may also attach to the platelets causing their destruction. Cytokines also cause destruction of the platelets. Inflammation and damage in various tissues of the body leads to platelet aggregation at the site of injury like endothelial injury. Decreased platelet count and lymphocyte count leads to higher PLR. Studies have shown a higher PLR in patients developing ARDS due to COVID-19 infection. Studies show that in SARS-CoV-2 infection, megakaryocyte may mature in lungs to increase local platelets and cause thrombocytopenia in blood circulation. Injury to lung parenchyma and endothelial cells can lead to activation and aggregation of platelets in

the lungs and thrombus formation in pulmonary vasculature. Finally the decreased blood supply distally may decrease the platelet generation and increase their consumption. Platelets have a role in haemostasis, coagulation, maintenance of vascular integrity; angiogenesis etc. local levels of platelets are found to be elevated within the pneumonic patches in basal lung fields. Variety of cytokines promotes the production of platelets from bone marrow. There is alteration in activity and no. of platelets in various infections. There are various markers which play crucial role in differentiation of megakaryocyte like stem cell factor promotes the proliferation of megakaryocytes, whereas interleukin and thrombopoetin act synergistically in differentiation therefore during any state of inflammation, there is increase in the level of Thrombopoetin which is induced by the IL-6 which promotes the generation of megakaryocytes. The patients in whom thrombocytopenia is found at the time of presentation are found to have a poor outcome as disease is likely to have progressed.

MPV (mean platelet volume) and PDW (platelet distribution width): Higher MPV and PDW suggest more activation of platelets and are found in various hyper coagulative disorders. Inflammation invidious tissues and organs of the body causes cytokine and chemokine production. The cytokines causes more activation of the platelets. Large platelets with high MPV contain more amounts of enzymes like thromboxane A2, thromboglobulin and platelet thrombofactor A, beta-thromboglobulin. The patients with higher level of MPV and PDW are found to have severe disease and higher risk of mortality [19,15].

Morphological changes: Peripheral blood smear showed patients with lymphopenia had few reactive lymphocytes with altered morphology, they appeared lymphoplasmacytoid. These cells have a distinctive abundant pale blue basophilic cytoplasm that often abuts adjacent red blood cells, eccentric nucleus and perinuclear clear halo denoting Golgi apparatus. Reactive lymphocytes with abundant cytoplasm scalloping around the other neighbouring cells giving rise to ballerina skirt appearance have been reported in various case reports. Russell bodies-plasma cells with abundant immunoglobulin inclusion bodies known as mott cells are also reported.

DISCUSSION

The opposite kinetics of lymphocytes and neutrophils is due to the depletion of CD8+ lymphocytes and simultaneous increase in interleukin-6,10,2 and interferon-gamma in severe patients as compared to those manifesting mild to moderate symptoms. Expansion of myeloid progeny with suppressor activity known as granulocyte myeloid suppressor cells takes place. These cells are immature and have a potent ability to suppress T-cell functions and modulate the cytokine production from inflammatory cells. The hyper-inflammatory response in COVID-19 induces an abnormal expansion of myeloid suppressor cells thus highly affecting the immunological response needed for clearance of the virus.

Thrombocytopenia has been found in both mild and severe disease, but mostly trend is towards normalisation of platelet count in the survivors. In non survivors and those with severe disease, platelets count are seen to be declining probably due to their increased production that is from megakaryocytic precursors and consumption at the site of infection that is the basal parts of lungs. Reduced production, increased destruction and enhanced consumption of platelets at the site of injury have been put forward.

Various studies shown that elevated neutrophil to lymphocyte ratio are an independent prognostic biomarker that causes progression of the lung injury in COVID-19 patients. As we know, neutrophil is a main inflammatory cell among leucocytes that gets activated and migrates from the circulation to the organ of inflammation. Neutrophils liberate huge amounts of reactive oxygen species which cause DNA damage and liberation of the virus from the host cells. Thus, antibody dependent cell mediated damage may kill the virus directly, expose viral antigen, and stimulate both the cell mediated and humoral immunity. Neutrophils interact with other immune cells to produce cytokines like VEGF. VEGF has a role in angiogenesis. Increased VEGF expression in COVID-19 patients is associated with lesser tissue and organ damage. Viral replication leads to generation of IL-6, IL-8, TNF-alpha, granulocyte colony stimulating factor, interferon-gamma from the endothelial cells and lymphocytes where viral proliferation takes place. Human immune response depends on the lymphocytes. The systemic inflammatory response inhibits the cellular immunity. Thus, CD8+ (suppressor) cells increase and CD4+ (helper) cells decrease. Thus NLR is increased in viral triggered inflammation. Elevated NLR leads to progression of COVID-19 disease. Elevated NLR was associated with increased severity from admission to ICU, mechanical ventilation was rapid. Neutrophil to lymphocyte ratio can be used as a red flag for deteriorating severe COVID-19 infection and can provide a firm base for early diagnosis and management of severe COVID-19 pneumonia.

According to a paper, a value of 2.8 for NLR and 180 for PLR seem to be suggestive for COVID-19 and eosinophils=0.15 [20].

CONCLUSION

COVID-19 is giving rise to tremendous challenges in the entire world and over pressurised the health care system and as of yet, efforts have been devoted to artificial intelligence based analysis of HRCT and x-rays have been used to indicate severity in the patients of SARS-CoV-2. This has aggravated the demand for radiologists and resulted in their shortage. To improve the diagnostic efficiency efforts should be made on a multi-diagnostic approach. Stress should be laid on categorising the patient according their clinical presentation and knowing their prognosis by different modalities like radiology,

pathology, virology so as to avoid burdening of a single modality of investigation. Moreover, CBC as we know is basic 1st line investigation in variety of diseases to know the immune status of the body.

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