Interleukin 6: The Best Marker for COVID-19 Disease Severity

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Abstract: *SARS CoV-2 has killed more than 60000 people in India alone till September 2020 and caused severe burden on health infrastructure of the country. There is a need for early and accurate predictors of severe infection at the early stage so that people can be triaged. Covid-19 presents with an array of symptoms ranging from asymptomatic to mild cough and cold to high grade fever and difficulty in breathing. There are many cases in which mild cases progressed to life threatening condition in a matter of a couple of hours. So, there is a need for a marker that can predict severity of the disease. Cytokines are synthesised in the body during infection and they can be used to predict severity of Covid-19 infection. In our study we are trying to prove IL-6 and IL-10 to be ideal candidates for prediction of Covid-19 disease severity.*

Keywords: COVID-19, SARS CoV-2, Cytokines, IL-6, IL-10.

1. Introduction

SARS CoV-2 is beta-corona virus that has caused COVID-19 pandemic which started from Wuhan, China and has killed 53,866 and infected 2.84 million people in India as of 20th August 2020.[1]It continues to pose an unprecedented challenge for the healthcare providers across the globe. Considering the high infectivity, rapid transmission and high fatality associated with COVID-19 infection; it is imperative to look for rapid tests to identify bio-markers that can predict the prognosis of the disease at an early stage so that patients can be triaged.[1]

Corona viruses are RNA viruses that are enveloped with positive sense RNA as genome. They are found in various species of the mammals and bats are known to harbour many types of the corona viruses. Previous to SARS in 2003 only 4 corona viruses were known to cause mild respiratory infections in the humans (229E, OC43, NL63, and HKU1). SARS-CoV2 is a beta corona virus related to theSARS and MERS corona viruses. Evidence from literature suggest that raised cytokine levels were the hallmark of these earlier corona virus diseases.[2] The word cytokine is derived from 2 words: Cyto means "cell" and kinos means "movement" in Greek. These are cell signalling molecules that help in cell communication, immune response and movement of immune cells towards the site of inflammation. Cytokine release syndrome was found to the major factor for mortality in patients infected with SARS-CoV and MERS and elevated levels ofIL-6 was considered as the hallmark of severe MERS CoV infection. Previous studies have highlighted that in SARS-CoV2 patients as well; cytokine storm is common in severe patients and is characterised by diffuse alveolar damage with hyaline membrane formation and infiltration of interstitial lymphocytes. Serum levels of various cytokines such as Interferon-gamma(IFN-γ), IL-6, IL-12 and IL-8 have been found to be raised in serious COVID-19 patients.Various recent evidences point to the fact that COVID-19 infection is characterized by an aggressive inflammatory response and release of pro-inflammatory cytokines in circulation. The immune response mounted by the host becomes hyperactive and results in excessive inflammatory reaction. Several studies have highlighted direct correlation between this cytokine storm and severity of COVID-19 diseases in the form of lung injury, multiorgan failure, and unfavorable prognosis.

The major threat associated with COVID-19 infection is the fatality associated with it as it has been found to be around 6.4%. In COVID-19 infection; ARDS and low oxygen saturation levels has been found to be major cause responsible for mortality. The exact mechanism of ARDS in COVID-19 patients is yet to be explored but it may be attributed to circulating pro-inflammatory cytokines. Hence early recognition of Cytokine storm in COVID-19 patients can aid clinicians in addressing not only the diagnostic challenges but also therapeutic challenges associated with COVID-19 infection. Drugs targeting these cytokines or their receptors may provide clinicians an opportunity to curb the morbidity and mortality associated with COVID-19 disease. This study aims to explore the potential use of various cytokines as prognostic factors for predicting the disease outcome/severity in COVID-19 patients timely and in an effective manner. Through this review; we aim to explore the usefulness of estimation of levels of various cytokines including IL-6 for predicting the probable clinical course amongst COVID-19 patients early on.[3]

2. Material and methods

Search strategy
A comprehensive literature search was performed on Pubmed, Dynamed, Google scholar, Cochrane databases for identification of articles on derangement of lab parameters in COVID-19 patients as per Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines. We used the following keywords for the search: COVID-19, Corona-virus, lab parameters, cytokines, IL-6, IL-10, Laboratory biomarkers, Laboratory parameters without date. A total of 163 studies were identified of which 14 were from Dynamed, 53 from google scholar, 25 on Pubmed and 71 were from various references. Our review included studies published between January 1- August 1, 2020. We then screened various articles on the basis of title, abstract and full text as per the search criteria:

Inclusion criteria for studies was:

- Lab parameters Y/N
- Comparison groups Y/N
- English Language Y/N
- Case series > 10 cases

- Articles on cytokines : IL-6, IL-10, TNF-α, Il-1, interleukins.

Case reports, review articles, letters, meta-analysis articles were excluded.

3. Results

After extensive screening of the articles we were able to identify only 25 studies describing various laboratory parameters in COVID-19 patients. Further after going through the full text of these articles, we were able to identify 20 articles on analysis of various cytokines including IL-6, IL-10, IL-1, IL-7 TNF-α in COVID-19 patients. (Table 1). Growing evidences from various studies suggest association of raised levels of inflammatory cytokines with worse prognosis in COVID-19 patients.

Interleukin 6(IL-6) was first described as a T cell-derived lymphokinewhose function was induction of final maturation of B cells intoantibody-producing cells. IL-6 is the main member of IL-6 superfamily. It is secreted by macrophages in response to microbial molecules which are recognised by receptors on the cells of the innate immune system called pattern recognition receptors(PRRs) recognising pathogen associated molecular patterns(PAMP) of microbes. These will in turn induce signalling pathways that will increase production of the inflammatory cytokines.[4] IL-6 also function as a potent stimulator of acute phase reactants secretion from the liver. Acute phase response is a systemic response against any inflammation, injury or infection. These acute phase reactant proteins are secreted from the liver and causes leucocytosis, increased vascular permeability, raised temperature. IL-6 induces secretion of several acute phase proteins such as fibrinogen, haptoglobin, serum amyloid A, C-reactive protein. Increased serum IL-6 also decreases secretion of albumin from the liver. Interferon-γ (IFN-γ) is the main cytokine of the Th1 T-helper cells. Th 1 is the immune response that kills intracellular parasites such as virus by cell mediated immunity. Apart from acute phase response IL-6 is also involved in B cell maturation, and differentiation of macrophages. IL-6 promotes Th2 differentiation and simultaneously inhibits Th1. IL-6 activates nuclear factor of activated T cells (NFAT) mediated transcription causing production of IL-4 by naive CD4 cells and their differentiation into Th2 cells. IL-6 inhibits Th1 differentiation by upregulating suppressor of cytokine signalling (SOCS)-1 expression thus interfering with IFN-γ signalling.[5] A Vultaggio et al in their study concluded that IL-6 levels were higher in in 62.9% of the patients and IL-6 was significantly associated with age. Kaplan-Meir survival plot was made by the authors and it showed that predictive performance of IL-6 at the time of admission for poor outcome of the disease. A statistical correlation was also found between IL-6 levels and ferretin, C-reactive protein (CRP), fibrinogen, white v blood cell (WBC) count and neutrophil count. Their conclusion was IL-6 along with CRP are among the best predictors of outcome of covid-19 disease.[6] According to the study done by Chomin Wu et al IL-6 levels in patients with acute respiratory distress syndrome(ARDS) who died were significantly higher than in patients with ARDS who survived and it was higher in inpatients with ARDS when compared with patients with no ARDS.[7]Maurizio Cecconi et al in Italy in their study concluded that IL-6 levels were significantly raised in patients with severe Covid-19 disease when compared too non-severe disease. Raised IL-6 along with lymphocyte count, Lactate dehydrogenase, D-dimer, creatinine, creatine kinase, ferretin and procalcitonin was associated with clinical deterioration and poor outcome of the disease.[8] Tie Long Chen et al n their study concluded that IL-6 levels were higher in Covid-19 patients. IL-6 levels in older patients (i.e. >65 years of age) were significantly raised when compared to younger patients.[9] Chuan Qin et al in China did a study on dysregulation of immune response in patients with coronavirus and found that severe inflammatory cytokines like IL-2R, IL-6, IL-8, IL-10 and TNF-alpha were significantly raised in severe cases compared to non-severe cases.[10]

Fei Zhou et al in their study in Wuhan found IL-6 levels significantly raised in non-survivors compared to survivors.[11] Michael Dreher et al in their study found that IL-6 levels in ARDS patients were raised significantly when compared with Non-ARDS patients. In their study IL-6 levels along with CRP, LDH, CK(creatine Kinase) and D-dimers were found to be raised.[12] Pingzheng Mo et al in their study tested IL-6 levels in patients of covid-19 after admission in the hospital. They divided patients into two groups refractory and general according to their clinical condition and found that Il-6 levels in refractory group was raised when compared to general group.[13] Ruchong Chen et al in their study found that IL-6 levels in Non-survivors were significantly higher when compared with survivors. IL-6 along with other parameters like CRP, Procalcitonin, Serum Amyloid A, ferritin and D-dimers were found to be raised in Non-survivors compared to survivors.[14] Xiochen Li et al found that severe patients of covid-19 had higher levels of IL-6 and TNF-α and a higher rate of ARDS compared to non-severe patients.[15] Yong Gao et al found that IL-6 and D-dimers combined were a sensitive marker for predicting severe Covid-19 disease. IL-6 levels were significantly raised in severe group. Area under curve(AUC) for IL-6 in ROC curve was 0.795 and IL-6 was found to be an independent risk factor for predicting ssevere covid-19 disease.[16] Kaiyan Li et al in ntheir study found out that various cytokines levels were significantly raised in Non-survivors when compared to survivors. These finding point out to a possible cytokine storm and were similar to findings of the previous SARS and MERS infection. Levels of IL-2R, IL-6, IL-8, Tumour Necrosis Factor-alpha(TNF-α) along with high sensitive CRP, procalcitonin were found to be significantly raised in non-survivor group.[17] Nanshen Chen et al in their study found out that IL-6 levels were raised in around 50% of the cases of covid-19.[18]Sufang Tian et al found out that levels of IL-6 and IL-10 were raised in all patients tested positive for Covid-19.[19] Tobias Herold et al in their study found out that median IL-6 levels were raised in patients requiring mechanical ventilation.[20] Matthew J Cummings et al in their study found out that IL-6 levels were raised in most of the patients od Covid-19 and established that Il-6 along with D-dimers were independent risk factor for in-hospital mortality.[21] Tau Liu et al in their study found out that baseline IL-6 levels were significantly raised in the severe patients when compared to the non-

severe. The increased IL-6 levels were closely related to the raised CRP, LDH, D-dimer levels. They also found out that lower the IL-6 levels shorter was the time taken to cure the disease in the patients and higher the IL-6 levels shorter the time taken from progression of the disease from onset to pneumonia.[22] According to the study done by Zhongliang Wang et al IL-6 levels were significantly raised in severe Covid-19 patients whose Sp0$_2$ levels were <90%.[23] Bo Diao et al in their study concluded that raised IL-6 levels along with raised IL-10 and TNF-α were negatively corelated with CD4+ T cell survival and that in-turn negatively corelated with increased morbidity in the covid-19 patients.[24] In a study done by Jing Gong et al ctyokines such as IL-6, IL-10, TNF-α along with CRP, ferroprotein and procalcitonin were found to be raised in severe patients of covid-19 when compared to mild ones.[25]

4. Discussion

Covid-19 infection causes rapid evolution of the disease into a severe form, therefore it is necessary to identify early predictors of the severe form of viral infection to identify and treat patients who are most prone for fatal disease. Serum SARS CoV-2 virus levels are closely related to IL-6 levels. IL-6 is produced by multiple types of cells including endothelial cells and production of IL-6 by the endothelial cells is reported to be the number one cause of cytokine release syndrome in the Covid-19 patients leading to pro-coagulant environment.[5] Corona virus infects immune cells like macrophage, dendritic cells and monocytes leading to their expression of IL-6. IL-6 through cis-signalling pathway acts on lymphocytes causes their Th-17 differentiation and through trans signalling pathway on endothelial cells causing increased secretion of other cytokines like VEGF, MCP and decreasing secretion of E-cadherin leading to recruitment of macrophages and vascular leakage. IL-6 through its action on hepatocytes causes increased secretion of acute phase protein such as CRP, SAA, hepcidin, fibrinogen, thrombopoietin, ferritin.[1] IL-6 has long been regarded as a marker of systemic pro-inflammatory cytokine activation. IL-6 has both pro and anti-inflammatory properties. IL-6 increases the production of glucocorticosteriods and downregulates synthesis of IL-1 and TNF along with other proinflammatory cytokines such as GM-CSF, IFN-γ and MIP-2. IL-10 is the main anti-inflammatory cytokine of the human body, it decreases Th-1 cytokines such as IL-2 and IFN-γ. IL-10 decreases synthesis of TNF-α, IL-1, IL-6, IL-8, IL-12, GCSF, MIP. Low concentration of IL-10 in lung may lead to the development of ARDS.[24] Many cytokines such as IL-6, TNF, IL-1, IL17, IL-2, interferon-γ, G-CSF(granulocyte colony stimulating factor), MCP-1(macrophage inflammatory protein-1) are found to be raised in severe patients in Covid-19. In our study we found out that IL-6 levels corresponds to the severity of the disease and it was found to be raised almost universally in every study in severe cases. Therefore, we present IL-6 as a single most important test for predicting outcome of the covid-19 at the time of admission. IL-6 was major cytokine during SARS outbreak that was raised and caused cytokine storm leading to fatality among severe cases. The cell mediated immunity and humoral immune system is known to be lower in old age and thus Th-2 response in predominant in the elderly make them prone for

the viral infection and higher fatality as seen during Covid-19 pandemic. Th-1 cytokines are responsible for anti-viral immunity of the body and thus cytokines such as IL-2R, IL-6, IL-8 and IL-10 are found to be raised in covid-19 patients.[15] Cytokine storm was first defined for graft versus host reaction in transplant cases and subsequently found to be a major pathogenic factor in various other diseases. IL-6 is a Th-1 cytokine that is released by many immune cells and it further recruits many cytokines to modulate immune response like decreasing the amount of typical Th-1 cytokines like IFN-γ and TNF-α. Multiple organ damage in the covid-19 patients is seemed to be mediated by Th-17 cells as cytokines related to Th-17 like IL-1, IL-17, TNF and GM-CSF were elevated in severe patients.[2] Apart from IL-6 other cytokines like anti-inflammatory IL-10 were also raised pointing towards ongoing tussle between inflammatory and anti-inflammatory responses inside the body Post- mortem examination in fatal patients revealed raised cytokines levels in lung tissue, cytokine levels were also raised in lymph nodes..[3] IL-6 is positively raised along with various acute phase reactants like CRP, Ferritin, Procalcitonin, LDH in many studies on covid-19.[5-10] Many studies have shown that IL-6 levels were significantly raised in severe Covid-19 cases when compared to non-severe cases. Low levels of IL-6 almost always rules out severe form of Covid-19 disease. Anti-IL-6 drugs (Tocizulumab) when used in severe covid-19 patients decreased the amount of cytokines as well as severity of the disease but it not still recommended for the mild-moderate disease as anti-viral activities of Th-1 response is responsible for clearing of the virus from the body and anti cytokine therapy when used improves disease outcome but prolongs virus clearing from the body.[23]

5. Conclusion

Il-6 is a pro-inflammatory cytokine that regulate immune response by recruiting various immune cells and by affecting synthesis of other inflammatory cytokines and acute phase reactant. Increased production of IL-6 can lead to a cytokine storm in the body that has detrimental effects in various organs of the body and can lead to fatality. Thus, IL-6 levels closely mimic severity of the covid-19 disease and can predict disease outcome early on.

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