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REVIEW ARTICLE

C- Reactive Protein (CRP) Test During COVID-19- An Important Marker in Prognosis of Severity

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ABSTRACT
The novel coronavirus (SARS CoV-2) is the reason behind the disease COVID-19 which has been declared pandemic by WHO and has spread to 227 countries. The disease has caused many deaths worldwide. Many bimolecular tests and biomolecules have been researched upon to find out possible biomarkers for prognosis of severity of COVID-19 disease in patients. From the site ofinflammation, macrophages, adipose tissue cells, lymphocytes and dendritic cells releases several proinflammatory cytokines which then leaks into blood stream and cause cytokine storm in body. This initiates a hyperinflammatory response and then to severe symptoms like single or multiple organ dysfunction and many a times a fatal outcome. Increased CRP (C-reactive protein) is often associated with death due to COVID-19 respiratory infection, as the infection progresses to lung lesions and inflammation of several organs (in later stages) leading to severe damage. In response to leakage of cytokines in blood stream, the liver releases several inflammatory biomarkers such as native or pentameric CRP which then binds at the lysophosphatidylcholine exposed by phospholipase A2 (PLA2) where pentameric CRP changes structural symmetry and dissociate to monomeric CRP which is highly proinflammatory. Human CRP molecules (pentameric CRP molecule and monomeric CRP molecule) have pleiotropic effect in human body on cells as it has properties of both anti-inflammatory and proinflammatory effects in human body. The present review explores the relationship of this significant protein with severity of illness during COVID-19 infection.
Keywords: *CRP, COVID, Corona virus, Pandemic, Cytokines, Severity, Proinflammatory, Anti-inflammatory*

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INTRODUCTION

Several proinflammatory cytokines which is leaked into the bloodstream when concentration is high, released by macrophages, dendritic cells, adipose tissue cells, lymphocyte etc. from the site of inflammation to blood[1]. The liver detects these proinflammatory cytokines and in response to that releases several inflammatory biomarkers and one of them is C-reactive protein (CRP).In its native form, it is pentameric in structure and is represented as pCRP. The protein is a homo-pentameric acute phase inflammatory biomarker protein andis a highly conserved plasma protein that was discovered in 1930 by Tillet and Francis while investigating the sera of patients suffering from the acute stage of *Pneumococcus* infection and was named for its reaction with the capsular (C)-polysaccharide of *Pneumococcus* **[**2]**.**

Transcriptional induction of CRP gene primarily occurs inside liver in hepatocyte cells and also low amount is released by smooth muscle cells, lymphocytes, endothelial cells, macrophages, and adipocytes [3]. CRP plays important role in inflammatory processes and host responses to infection including the classical complement pathway, apoptosis, phagocytosis, nitric oxide (NO) release, detoxification of toxic substance released from damaged cells and the production of cytokines, particularly interleukin-6(IL-6) and tumor necrosis factor-α(TNF-α) (Figure 1). CRP may rise within 6-8 hours after the onset of

inflammation and is the most sensitive non-specific indicator of inflammation inside body. It is both proinflammatory and anti-inflammatory in nature.

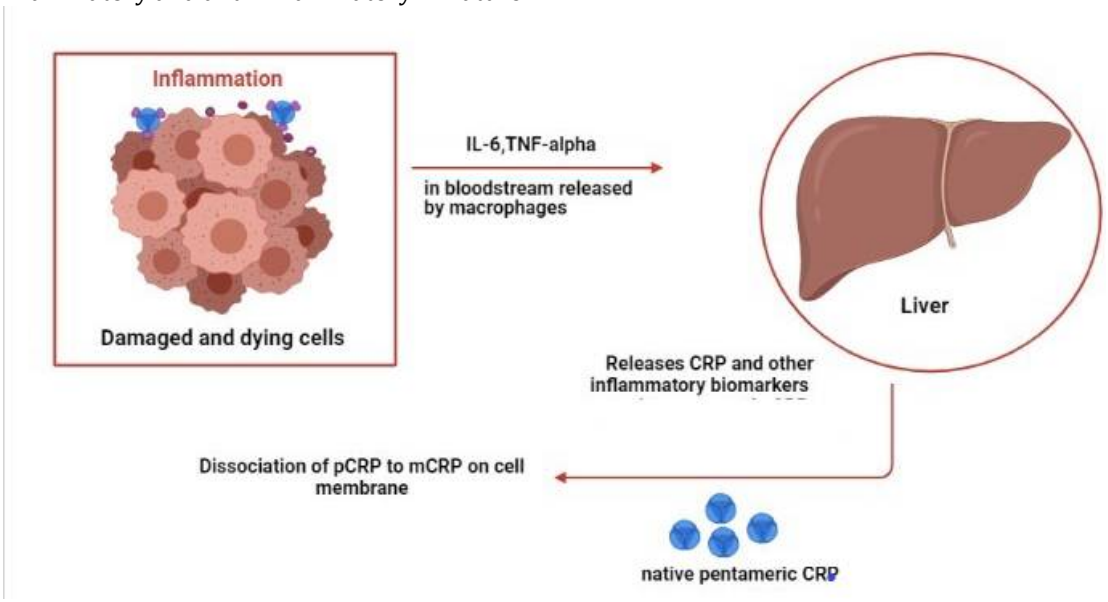

Figure 1: Induction of CRP by cytokines from liver

CRP is released from mainly in response to cytokines like IL-6, IL-1β and sometimes IL-8 and TNF-α[3]. The higher the concentration of these cytokines is present in bloodstream, the more amount of pCRP is released by the liver in response to that. Number of studies have reported correlation of CRP levels with disease prognosis, such as in patients with atherosclerotic disease, myocarditis, aortic valve disease, congestive heart failure and heart transplantation, indicating an active and significant role of this protein in the physiology of cardiovascular disease [4]. Increased levels of CRP have also been found in patients withpancreatitis, appendicitis, cholecystitis, pelvic inflammatory disease, gout, stroke, urinary tract infection, pneumoniaand meningitis[5].

Structure and phylogeny of C-reactive protein

CRP is a homopentameric protein belonging to pentraxin family of calcium-dependent ligand binding plasma proteins. Human pCRP consist of five identical subunits. It is composed of 206 amino acids and has half-life of 19-24 hours. Each subunit or protomers are bonded noncovalently forming cyclic pentameric structure. Human CRP has phosphocholine (PC) binding site which have high affinity towards phosphocholine residues which is present on dead or damaged cells but can bind to other autologous and extrinsic ligands.On the other hand, extrinsic ligands include many glycan, phospholipid, and other constituents of microorganisms, such as capsular and somatic components of bacteria, fungi, and parasites, as well as plant products. When aggregated or bound to macromolecular ligands, human CRP is recognized by C1q and activates the complement pathway [3]. Studies shows that on the basis of structure and ligand binding specificity of CRP to phosphocholine and related molecules, phlylogenetic conservation of CRP molecule and absence of any protein deficiency or protein polymorphism suggests that this protein molecule has survival value and microbial diseases are major driving force of change in course of evolution in organisms and CRP molecule have many features compatible with a role in innate immunity[6].

Isoforms of CRP

CRP molecule exists in majorly two forms in humans which are pentameric C-reactive protein (pCRP) and monomeric C-reactive protein (mCRP). The pCRP molecules are native CRP released from liver hepatocytes (Figure 2) in response to proinflammatory cytokines like IL-6, IL-8 and TNF-α etc, in bloodstream which at site of inflammation binds to phosphocholine residues(1,6 Bis (phosphocholine)-hexane), lysophosphatidylcholine on cell surface which is induced by the action of Phospholipase A2 (PLA2) [7].

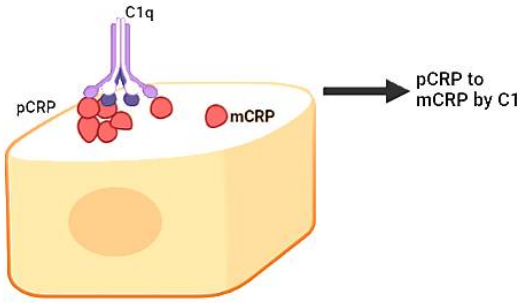

Figure 2: Activation of pCRP to mCRP

When pCRP is bound to the cell surface by PC binding site, molecule C1q recognizes pCRP and binds through its globular head domain thus leading to C1q mediated classical complement pathway activation. pCRP after binding to cell membrane and C1q, undergoes partial structural change and then loose pentameric symmetry leading to the formation of mCRP which is a highly proinflammatory[3]. As mCRP is formed only at the site of inflammation, it is present in higher concentration on the site of inflammation rather than in blood which implies that in hs-CRP higher concentration of pCRP is detected rather than mCRP. CRP then stimulates classical complement pathway which leads to phagocytosis, opsonization and lysis of apoptotic cells, necrotic cells, dead cells and pathogens. Figure 3 explains the events leading to activation of complement pathway.

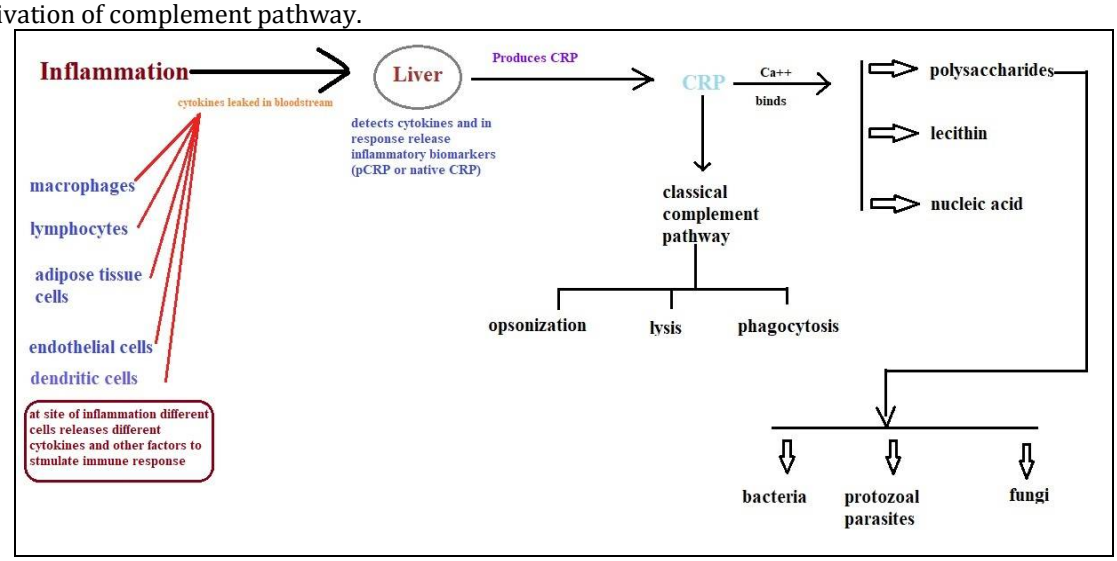

Figure 3: Role of CRP in classical complement pathway

Glycosylated CRP

Glycosylated CRP is formed in some rare diseases. According to Das et al.[8]pentameric structure of CRP is stable and it was highly unlikely for CRP to form a glycosylated compound but in some diseases like systemic lupus erythematosus, acute lymphoblastic leukaemia, tuberculosis, visceral leishmaniasis, osteogenic sarcomaand Cushing's syndrome,the presence of sugars such as glucose, mannose,sialic acid,galactose have been demonstrated in CRPs, and revealed differences in CRP carbohydrate and amino acid composition between different samples[9,10]. In these diseases, the glucose is bound to the oxygen and nitrogen forming O- and N-linked glycoproteins to induce hemolysis of erythrocytes in the human body [11].

Interpretation of CRP level in human body

High sensitivity CRP test (hs-CRP test) is a standard quantitative test to markedly measure the level of CRP in the body; high sensitivity CRP test can detect even a slight change in concentration of CRP in blood. The hs-CRP test is two reagent immunoturbidimetric methods. The circulating CRP in sample leads to formation of immune complex which increase the scattering of light implying the scattering of light is directly proportional to CRP concentration in a sample. The absorbance of light due to this scatter of light is measured in spectrophotometer. Turbidity is measured at a primary wavelength of 546 nm (secondary wavelength 800 nm). The light absorbance observed is read against the standard CRP curve. Following interpretation is made by CRP concentration measured in serum(Table-1)[2].

Table 1 : Interpretation of concentration of CRP levels

Range	Interpretation of causes of elevation
Less than 0.3 mg/dL	Normal (level seen in most healthy adults).
0.3 to 1.0 mg/dL	Normal or minor elevation (can be seen in obesity, pregnancy, depression, gingivitis, diabetes, sedentary lifestyle, cigarette smoking, common cold, and genetic polymorphisms).
1.0 to 10.0 mg/dL	Moderate elevation (Systemic inflammation such as rheumatoid arthritis, Systemic lupus erythematosusor other autoimmune diseases, myocardial infarction, pancreatitis, bronchitis, malignancies,).
More than 10.0 mg/dL	Marked elevation (Acute bacterial infections, viral infections, systemic vasculitis and major trauma).
More than 50.0 mg/dL	Severe elevation (Acute bacterial infections).

According to the table a rough range of CRP levels in viral diseases lie above 100mg/L but according to some studies the concentration of CRP released highly depends upon the individual. The factors affecting the CRP levels are vast like gene makeup of an individual, immune response of an individual, age, autoimmune diseases and other co-morbidities, diabetes, liver ailments (because of alcohol), liver damage and liver diseases[12,13].According to study by Nehring *et al*. [2], CRP has been used as a prognostic factor in acute and chronic infections whether it is viral or bacterial infections, including dengue, hepatitis C, and malaria. About 90% of the time, very high level of CRP (more than 50 mg/dL) is associated with bacterial infection.

Studies show that there are vast numbers of factors affecting CRP levels in human body. There are several diseases of the category of infection, malignancy, rheumatologic, drug reactions, autoimmunity, multiple organ disease, gastrointestinal diseases, liver diseases (like COPD- chronic obstructive pulmonary disease) which leads to higher peaks of CRP concentration in blood[14,15]. Medications like statins, non-steroidal anti-inflammatory drugs (NSAID's) and magnesium supplements may falsely reduce CRP concentration[16,17]. Also, depression, obesity, insomnia can mildly elevate CRP levels in body[**Error! Reference source not found.**]. About 90% of time, when patient's blood sample had CRP concentration more than 50 mg/dL it was because of bacterial infection. It is hard to interpret mild elevation in CRP levels, especially in range of 1.0 mg/dL to 10 mg/dL[18].

Role of CRP in prognosis of severity in COVID-19 patients

In a study conducted from January 23, 2020 to February 29, 2020 on COVID-19 patients admitted in hospital, where CT scan of patients with largest lung lesions and from mild cases were compared and also their CRP tests were done which showed significantly higher level of CRP of severe COVID-19 patients than mild or moderate cases. Though the study was done on smaller population without the knowledge of co-morbidities of the patients, according to this, the CRP levels are not affected by age of patients in case of COVID-19 infection [19].

It was also noted that one unit increase of CRP in patients' blood sample had led to 5% more probability of severe events. It was also observed in several studies that more than 80% severe patients showed elevated level of CRP[20,21, 22, 23, 24].According to Wu & Shen's latest report, the sensitivity of COVID-19 diagnosis with CT scan alone was 76.4%, and the application of CT scan in COVID-19 was evaluated as useful [25]. Severe cases had significantly higher level of CRP concentration than non-severe or mild cases. Also, mild cases had much lesser mean CRP levels (23 mg/L) than severe patients (46 mg/L) [20]. Table 2 below shows the available studies where CRP concentrations are determined in several cases during the ongoing pandemic.

It was also seen that patients had higher CRP levels (76.5 mg/L median) when low oxygen saturation level was observed during diagnostic which was SpO2 lower or equal to 90% but mild cases which had reading of SpO2 level higher than 90% had CRP level around (12.7 mg/L median)[20].

High concentration of CRP levels may be linked to higher cytokine concentration in bloodstream. Elevate cytokines helps patient's body to fight microbes but sometimes in some patients, immune response goes out of control and leads immune system to get hyperactive which leads to cytokine storm in body which can further damage lungs and other organ dysfunction which leads to sepsis and death [34]. The CRP levels in early cases can be used to determine the condition of patients and can predict the severe events if compared to other clinical findings like CT scan of lungs which can help in ruling out the other co-morbidities which can be present in several patients.

Table 2: Levels of C-reactive protein (CRP) in patients with COVID-19

Group	Patients (n)	CRP, mg/L	*P* value	N and % of patients with elevated CRP	Reference
Hospitalized	99	51.4 (41.8)	NA	63/73 (86)	[21]13
Death	113	113 (69.1-168.4)	NA	59/68 (60)	[22]22
Recovered	161	26.2 (8.7-55.4)		21/45 (14)	
Severe	15	39.4 (27.7)	0.011	NA	[23]23
Mild	28	18.8 (22.2)			
Severe	173	NA	NA	110/135 (81.5)	[26]24
Non-severe	926	NA		371/658 (56.4)	
Severe (GI symptoms)	74	15.7 (4.8-23.9)	0.003	NA	[27]25
Non-severe (no-GI symptoms)	577	7.9 (2.6-19.6)			
Severe	13	62.9 (42.4-86.6)	NA	NA	[28]28
Mild	27	7.6 (3.1-57.3)			
Died	84	100 (60.7-179.4)	0	NA	[29] 29
Recovered	214	9.6 (5-37.9)			
Severe	85	46 (22-106)	0.001	NA	[24] 24
Mild	70	23 (10-47)			
Severe	139	43.1 (9.8-97.3)	<.001	NA	[30]30
Non-severe	304	10 (2.9-27.1)			
Hospitalized	81	47.6 (41.8)	NA	NA	[31]31
Severe	16	43.8 (12.3-101.9)	0	NA	[32]32
Non-severe	193	12.1 (0.1-91.4)			
Severe	6	65.6 (47.5-97.5)	NA	NA	[33]33
Non-severe	18	11.1 (0.9-19.1)			

CONCLUSION

CRP is a remarkable biomarker for early patients for the prognosis of future severe events of patient and to treat them accordingly based on the levels of CRP in blood sera of patients. hs-CRP test is suggested than standard CRP test as hs-CRP shows even lower levels of CRP concentration and will allow us to detect even slight change in CRP concentration changes. Detection of CRP levels in early patients could be helpful in prediction of severity of condition in patients after taking account of the other co-morbidities patient maybe suffering since past also mild elevations in patients with co-morbidities can lead to false interpretations without other methods of detection of COVID-19 infection level in patients. Also, to be more precise it may be advisable to conclude the patient condition and predict future condition of patient based on several clinical diagnostics including CRP level of the patient.

REFERENCES

1. Zhang, J. M., & An, J. (2007). Cytokines, inflammation, and pain. International anesthesiology clinics, 45(2), 27–37. https://doi.org/10.1097/AIA.0b013e318034194e
2. Nehring SM, Goyal A, Bansal P, et al.(2021 Jan) C Reactive Protein. [Updated 2021 May 10]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov /books/NBK441843/
3. Sproston, N. R. and Ashworth, J. J.(2018) Role of C-Reactive Protein at Sites of Inflammation and Infection. Frontiers in Immunology., 9:754. https://doi.org/10.3389/fimmu.2018.00754
4. Osman R, L'Allier PL, Elgharib N, Tardif JC.(2006) Critical appraisal of C-reactive protein throughout the spectrum of cardiovascular disease. Vasc Health Risk Manag.;2(3):221. doi:10.2147/vhrm.2006.2.3.221.
5. Yu-Jang Su, The value of C-reactive protein in emergency medicine, Journal of Acute Disease, Volume 3, Issue 1,2014,Pages 1-5,ISSN 2221-6189,https://doi.org/10.1016/S2221-6189(14)60001-9.
6. Pepys, M. B. and Hirschfield, G. M.(2003) C-reactive protein: a critical update. J Clin Investig; 111(12):1805–1812. https://doi.org/10.1172/JCI18921
7. Thiele, J. R., Zeller, J., Bannasch, H., Stark, G. B., Peter, K., & Eisenhardt, S. U. (2015). Targeting C-Reactive Protein in Inflammatory Disease by Preventing Conformational Changes. Mediators of inflammation., 372432. https://doi.org/10.1155/2015/372432
8. Das T., Sen A.K., Kempf T., Pramanik S.R., Mandal C., Mandal C. Induction of glycosylation in human C-reactive protein under different pathological conditions. *Biochem. J.* 2003;373:345–355. doi: 10.1042/bj20021701.

9. Ansar, W., Mukhopadhyay, S., Habib, S.H. et al. (2009) Disease-associated glycosylated molecular variants of human C-reactive protein activate complement-mediated hemolysis of erythrocytes in tuberculosis and Indian visceral leishmaniasis. Glycoconj J 26:1151-1169. https://doi.org/10.1007/s10719-009-9236-y
10. Boncler, M., Wu, Y., &Watala, C. (2019) The Multiple Faces of C-Reactive Protein-Physiological and Pathophysiological Implications in Cardiovascular Disease. Molecules(Basel, Switzerland), 24(11), 2062. https://doi.org/10.3390/molecules24112062
11. Sola R.J. and GriebenowK.(2010) Glycosylation of therapeutic proteins: An effective strategy to optimize efficacy. BioDrugs.,24:9–21. doi: 10.2165/11530550-000000000-00000.
12. Chen L, Deng H, Cui H, et al. (2018) Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 9(6):7204. doi:10.18632/ONCOTARGET.23208
13. Marjot T, Webb GJ, Barritt AS, et al.(2021) COVID-19 and liver disease: mechanistic and clinical perspectives. Nat Rev Gastroenterol Hepatol;18(5):348-364. doi:10.1038/s41575-021-00426-4
14. Jain S, Gautam V, Naseem S. (011) Acute-phase proteins: As diagnostic tool. *J* Pharm Bioallied Sci;3(1):118. doi:10.4103/0975-7406.76489
15. Landry A, Docherty P, Ouellette S, Cartier LJ.(2017) Causes and outcomes of markedly elevated C-reactive protein levels. Can Fam Physician. 63(6):e316. Accessed July 26, 2021. /pmc/articles/PMC5471098/
16. Prasad K. (2006) C-reactive protein (CRP)-lowering agents. Cardiovasc Drug Rev., 24(1):33-50. doi:10.1111/J.1527-3466.2006.00033.X
17. Tarp S, Bartels EM, Bliddal H, et al. (2012) Effect of nonsteroidal anti-inflammatory drugs on the C-reactive protein level in rheumatoid arthritis: a meta-analysis of randomized controlled trials. Arthritis Rheum., 64(11):3511-3521. doi:10.1002/ART.34644
18. Lelubre C, Anselin S, ZouaouiBoudjeltia K, Biston P, Piagnerelli M. (2013) Interpretation of c-reactive protein concentrations in critically Ill patients. Biomed Res Int.;2013. doi:10.1155/2013/124021
19. Wang L. (2020) C-reactive protein levels in the early stage of COVID-19. Medecine et Maladies Infectieuses., *50*(4), 332–334.https://doi.org/10.1016/j.medmal.2020.03.007
20. Ali N. (2020). Elevated level of C-reactive protein may be an early marker to predict risk for severity of COVID-19. *Journal of Medical Virology*, *92*(11), 2409–2411. https://doi.org/10.1002/jmv.26097
21. Chen N, Zhou M, Dong X, et al. (2020a) Epidemiological and clinical characteristics of 99 cases of 2019novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 395(10223):507-513. 10.1016/S0140-6736(20)30211-7
22. Chen T, Wu D, Chen H, et al. (2020b) Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. BMJ.;368 10.1136/bmj.m1091
23. Gao Y, Li T, Han M, et al. (2020) Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID-19. J Med Virol. 10.1002/jmv.25770
24. Mo P, Xing Y, Xiao Y, et al. (2020) Clinical characteristics of refractory COVID-19 pneumonia in Wuhan, China. Clin Infect Dis: 10.1093/cid/ciaa270
25. Wu Jn, Shen J. (2020) Emphasis and scientific evaluate the role of CT in the diagnosis and treatment of novel coronavirus pneumonia. J Dalian Med Univ,.42(01):1–4. https://kns.cnki.net/KCMS /detail/43.1390.R. 20200310.0937.002.html
26. Guan WJ, Ni ZY, Hu Y, et al. (2019) Clinical characteristics of coronavirus disease in China. N Engl J Med. 2020;382(18):1708-1720. 10.1056/NEJMoa2002032
27. Jin X, Lian J-S, Hu J-H, et al.(2019) Epidemiological, clinical and virological characteristics of 74 cases of coronavirus-infected disease (COVID-19) with gastrointestinal symptoms. Gut. 2020;69(6):1002-1009. 10.1136/gutjnl-2020-320926
28. Liu J, Li S, Liu J, et al. (2020) Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. EBioMedicine.;55:102763 10.1016/j.ebiom.2020.102763.
29. Wang S, Lin D, Yang X, et al. (2020b) Prognostic value of C-reactive protein in patients with COVID-19. Infect Dis., 9:2445-2453. 10.1101/2020.03.21.20040360
30. Shang W, Dong J, Ren Y, et al. (2020) The value of clinical parameters in predicting the severity of COVID-19. J Med Virol., 10.1002/jmv.26031 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
31. Shi H, Han X, Jiang N, et al.(2020) Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: A descriptive study. Lancet Infect Dis., 20(4):425-434. 10.1016/S1473-3099(20)30086-4
32. Wang G, Wu C, Zhang Q, et al. (2020a) C-reactive protein level may predict the risk of COVID-19 aggravation. Open Forum Infect Dis., 7(5). 10.1093/ofid/ofaa153
33. Young BE, Ong SWX, Kalimuddin S, et al. (2020) Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. JAMA., 323(15):1488 10.1001/jama.2020.3204
34. Sinha P, Matthay MA, Calfee CS.(2020) Is a "Cytokine Storm" Relevant to COVID-19? *JAMA Intern Med.*, 180(9):1152–1154. doi:10.1001/jamainternmed.2020.3313.