Diagnostic significance of serum biomarkers in SARS-CoV-2 infections: study findings

By Reena Rajan

Type of manuscript: Original article

Diagnostic significance of serum biomarkers in SARS-CoV-2 infections: study findings

Reena Rajan[1], Priya K Dhas[2], S. Mathavi[1]

[1]Department of Microbiology, Vinayaka Mission's Kirupananda Variyar Medical College & Hospitals, Vinayaka Mission's Research Foundation (Deemed to be University) Salem, Tamil Nadu, India

[2]Department of Biochemistry, Vinayaka Mission's Kirupananda Variyar Medical College & Hospitals, Vinayaka Mission's Research Foundation (Deemed to be University) Salem, Tamil Nadu, India

Corresponding Author: Reena Rajan

E-mail address: reenarajan83@gmail.com

ABSTRACT

Background. The clinical relevance of serum biomarkers plays a critical role in Covid-19 management. This study aimed to evaluate the role of serum biomarkers in the early detection of clinically suspected Covid-19 cases.

Methods. A retrospective analysis was conducted on 113 clinically suspected SARS-CoV-2 cases. The study groups were divided into two based on the Reverse Transcriptase Polymerase Chain Reaction (RTPCR) test result. The severity of illness was assessed for both the study groups by Chest Computed Tomography severity score. Data on serum biomarkers such as D Dimer, C reactive protein, Ferritin and Lactate dehydrogenase was evaluated for the study groups. Analysis of data was done by Chi-square test; p value of less than 0.05 was taken as statistically significant.

Results. Increased C reactive protein was observed among 94(83.19%) cases, 32 (28.32%) patients showed abnormal LDH. Elevated D dimer was observed in 20/113(17.70%) and serum ferritin in 77/113(68.14%) cases. No statistically relevant correlation (p>0.05) found between serum markers and disease severity. No statistically relevant association (p value more than 0.05) observed among RTPCR positive and negative study groups and serum biomarkers.

Conclusion. Serum biomarkers aids in supporting the diagnosis of Covid-19 in clinically suspected cases. We have evaluated the role of serum biomarkers and its association with mild to moderate and severe Covid-19. No significant association was found between elevated serum biomarkers and mild to moderate and severe Covid-19. Decreased levels of serum biomarkers was observed in patients who recovered from severe infection.

Keywords: Covid-19, Severity, Serum biomarkers, RTPCR, Chest CT scan

INTRODUCTION

The susceptibility to COVID-19 primarily depends on surface glycoprotein affinity to Co-receptor in target tissues [1]. The binding of the SARS Cov-2 spike protein to Angiotensin Converting Enzyme 2 has been suggested to cause Angiotensin Converting Enzyme -2 downregulation from the host cell membrane which leads to a loss of protective effects by Angiotensin receptor-1(ARB) and Angiotensin converting enzyme inhibitors (ACEi) in humans [2]. Clinical manifestation of SARS-CoV-2 varies from asymptomatic carrier stage to patients requiring admissions in intensive care units and assisted ventilator support [3]. SARS-CoV-2 get attached to alveolar epithelial cells and activates native and adaptive immune response, which results in the release of multieffective cytokine, interleukin-6. Elevated IL-6 mediates cytokine storm causing tissue injury and Covid-19 progression. Elevated IL6 levels in Covid-19 patients is associated with respiratory distress syndrome, secondary infections and death [4].

Inflammatory changes in the lung, platelets recruitment with degranulation, clot formation, altered vessel permeability and leukocyte accumulation in the injury site leads to recruitment of IL-4, IL-13, transforming growth factor β which is responsible for profibrotic activity.

The mechanism underlying multisystem involvement in Covid-19 patients may include an abnormal immune response induced by enhanced concentration of proinflammatory mediators such as interleukins, IL-1 β, IL-2, IL-6, IL-8, interferon induced protein -1 alpha and tumor necrosis factor alpha [5]. Lactate dehydrogenase (LDH) enzyme, C reactive protein (CRP), and Ferritin are some of the serum biomarkers responsible for the hyper inflammatory response and multi organ failure in Covid patients [6].

The level of C reactive protein elevates following secretion of IL-6 by macrophages and T cells. Increased CRP levels is directly related to the level of inflammation, severity of disease and development of lung lesions in initial stage of Covid-19 [7]. In severe disease, elevated levels of D dimer indicate clotting alterations. Patients with increased D dimer levels require intubation and are linked with enhanced probability of developing a pulmonary embolism. Low platelet level has been related to increased risk of severe COVID-19 and mortality and can serve as an indicator of worsening of clinical disease during hospitalisation. In prolonged Covid-19 clinical condition, elevated Ferritin and long-lasting inflammatory effects can contribute to multiple organ disease [8]. Severe Covid-19 infection causes tissue damage and subsequent release of LDH into the circulation results in severe interstitial pneumonia [9].

Understanding early biomarkers for Covid-19 prognosis may aid to prevent virus induced acute inflammatory response complications such as acute hepatic, cardiac and renal injury in affected patients. Due to Covid-19 disease's novelty, the criteria that influences the severity of the disease condition and mortality remains unclear. There is only a limited data available regarding sensitivity and specificity of laboratory test in determining Covid-19 disease severity and available literature showed that the most appropriate biomarkers in Covid-19 severe cases have yet to be defined. Hence this study was undertaken to investigate the role of biomarkers in assessing disease severity and outcome in Clinically suspected laboratory confirmed SARS-CoV-2 cases.

MATERIALS & METHODS

A retrospective analysis was done on Covid-19 patient's data collected from the month of April 2021 to May 2021. The study subjects who were admitted at VMKV

Medical College & Hospitals, Salem, Tamil Nadu were categorised into two based on the clinical characteristics, CT findings and molecular testing result.

Group I: Patients with clinically suspected SARS-CoV-2 infection and tested positive by Reverse Transcriptase Polymerase Chain Reaction.

Group II: Patients with clinically suspected SARS-CoV-2 infection and tested negative by Reverse Transcriptase Polymerase Chain Reaction.

As per instruction of manufacturer, Real Time Polymerase Chain Reaction assay was performed on Quanstudio 5 (Thermofischer scientific) by using COVID sure multiplex RT-PCR kit, based on the analysis of 3 fluorochromes, FAM (Orf 1ab gene), HEX (E gene) and ROX (Internal control)

Patients were divided into mild (CT severity score ranging from 0 to7), moderate (CT severity score ranging from 8 to 14) and severe (CT severity score ranging from 15 to 25) category based on chest CT findings.

Laboratory parameters such as C - reactive protein (Reference range: ≤10mg/L), LDH (Reference range:30-1000 IU/L), D-dimer (Reference range :<500ng/ml) and serum Ferritin (Reference range; Male:68-434 ng/ml; Female 24.4-278 ng/ml) etc were assessed for both the study groups. Serum biomarkers were measured using standard calorimetric and turbidometric immunoassay. Chi-square test (p value of less than 0.05 was considered as statistically significant) was used to analyse data.

RESULTS

Among 113 patients studied, more than 60% patients were younger than 60 years and majority of the participants in the study cohort were males 77 (68.14% male vs. 36 (31.86 %) females). [Fig:1] In Group I category,25(64.10%) patients were males and 14(35.90%) were females. In Group II category,52(70.27%) were males and 22(29.73%) were female patients. [Table:1] 28/113(24.78%) patients had severe infection, 49(43.36%) had moderate infection and 36(31.86%), showed mild disease. Out of 28 patients with severe disease, 11(39.29%) were females and 17(60.71%) were males. Mild to moderate infection was observed among 85 patients,60(70.59%) males vs. females 25(29.41%) [Table: 2]

Ninety-four (83.19%) cases showed elevated biomarker C- reactive protein (Group I :31.91% vs Group II:68.09%). Thirty two (28.32%) patients showed abnormal LDH (Group I :46.88% vs Group II:53.52%) Elevated D dimer was observed among 20/113(17.70%) patients (Group I: 35.00% vs Group II :65.00%). Elevated serum

Ferritin was observed among 77/113(68.14%) cases (Group I: 28.57% vs Group II: 71.43%) [Table :1]

An increased level of CRP was observed among 23(24.47%) severe cases versus 71 (75.53%) non severe cases. Elevated LDH was found among 12(37.50%) severe cases versus 20(62.50%) non severe cases. Ten (50.00%) each showed elevated D dimer among the severe cases and non severe cases. Elevated Ferritin level was observed among 20(25.97%) severe cases and 57(74.03%) non severe cases [Table: 2]
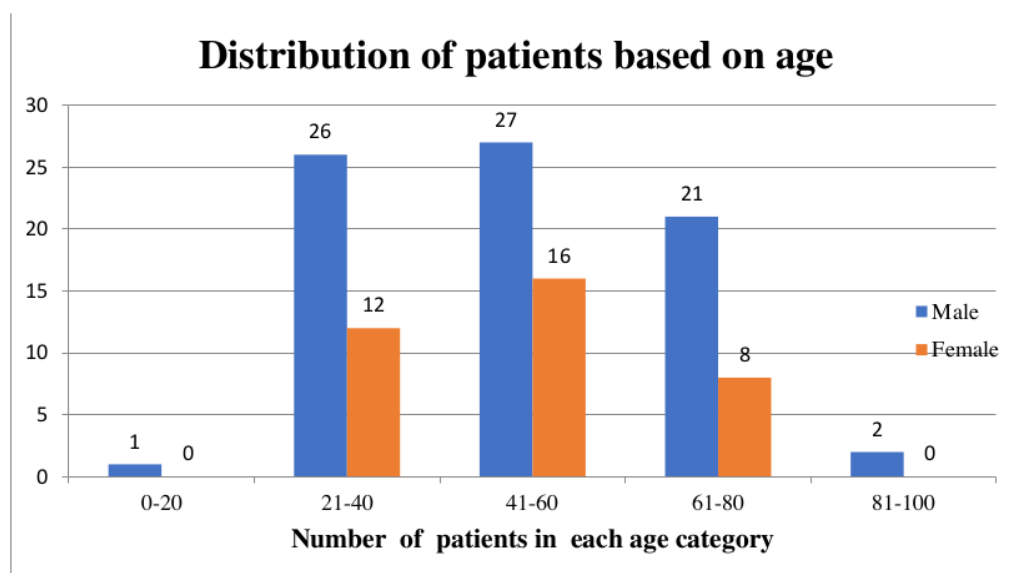

Distribution of patients based on age

Fig 1: Sociodemographic characteristics of Covid-19 patients

No statistically relevant association (p>0.05) was observed between elevated serum biomarkers and RTPCR positive and negative study groups. Similarly, there was no statistically relevant correlation (p value of more than 0.05) found between elevated serum markers and Covid 19 disease severity.

Table: 1-Comparison between RTPCR assay and elevated biomarkers in Covid-19 patients.

Biomarkers		RTPCR		P-value
		Negative	Positive	
CRP	Elevated	64 (68.09)	30 (31.91)	0.196
	Normal	10 (52.63)	9 (47.37)	

LDH	Elevated	17 (53.12)	15 (46.88)	0.082
	Normal	57 (70.37)	24 (29.63)	
D-dimer	Negative	6 (42.86)	8 (57.14)	0.201
	Positive	13 (65.00)	7 (35.00)	
Ferritin	Elevated	55 (71.43)	22 (28.57)	0.052
	Normal	19 (52.78)	17 (47.22)	
Sex	Female	22 (61.11)	14 (38.89)	0.504
	Male	52 (67.53)	25 (32.47)	

Table: 2-Comparison between severity of lung involvement and elevated biomarkers in Covid-19 patients

Biomarkers		CT Severity			P-value
		Mild	Moderate	Severe	
CRP	Elevated	30 (31.91)	41 (43.62)	23 (24.47)	0.985
	Normal	6 (31.58)	8 (42.11)	5 (26.32)	
LDH	Elevated	8 (25)	12 (37.5)	12 (37.5)	0.140
	Normal	28 (34.57)	37 (45.68)	16 (19.75)	
D-dimer	Negative	4 (28.57)	6 (42.86)	4 (28.57)	0.413
	Positive	5 (25)	5 (25)	10 (50)	
Ferritin	Elevated	22 (28.57)	35 (45.45)	20 (25.97)	0.548
	Normal	14 (38.89)	14 (38.89)	8 (22.22)	
Sex	Female	13 (36.11)	12 (33.33)	11 (30.56)	0.327
	Male	23 (29.87)	37 (48.05)	17 (22.08)	

DISCUSSION

SARS-CoV-2 is a rapidly expanding pandemic increasing health care facilities burden. It is crucial to monitor patient conditions in a timely manner to predict Covid-19 related complications. Identification of biomarkers helps to understand the mechanism of viral pathogenesis as well as cellular and organ damage. While assessing patients, biomarkers can be useful for clinical management and prevention of serious complications in Covid-19 [10].

In our study, majority of the patients (>60%) were younger, in the age group of ≤60 years and were males (68.14% male vs. 31.86 % females). 28(24.78%) patients had severe infection and 85(75.22%) had mild to moderate infection.18/28(64.29%) severe cases were in the category of 21-60 years. In contrast to present study finding, an increased severity of Covid-19 disease among elderly patient when compared to those who were young and middle-aged have been reported [11]. Prolonged hospital stay and significantly high level of serum CRP, D dimer and Ferritin levels among elderly patients when compared to young and middle age groups have been reported [12].

In the present study, 94 (83.19%) cases showed elevated C reactive protein and 32(28.32%) patients showed abnormal LDH. Elevated D dimer was observed among 20/113(17.70%) patients and increased serum Ferritin was observed among 77/113(68.14%) cases. There are study reports on the role of blood biomarkers as promising diagnostic tool for Covid-19 disease progression [13].

Table:3 Studies on the role of serum biomarkers as diagnostic tool for Covid-19

Various Studies	Biomarkers Analysed	Study Findings in Covid cases

Das B et al [14]	C-reactive Protein	Increased (79%)
	Ferritin	Increased (56%)
	D Dimer	Increased (80%)
	LDH	Increased (42%)
Chen L et al [15]	C-reactive Protein	Increased (93.10%)
	LDH	Increased (68.97%)
Liu Y et al [16]	D Dimer	Increased (17.00%)
Poudel A et al [17]	D Dimer	Increased (69.23%)
Yao Y et al [18]	D Dimer	Increased (74.60%)
Alroomi M et al [19]	Ferritin	Increased (67.69%)
Present study	C-reactive Protein	Increased (83.19%)
	Ferritin	Increased (68.14%)
	D Dimer	Increased (17.70%)
	LDH	Increased (28.32%)

Elevated C reactive protein is linked with overproduction of inflammatory cytokines which is also related to severity and patient mortality in Covid patients. We have evaluated the role of elevated CRP level and its association with severity of Covid 19. In the current study, twenty three (24.47%) severe cases had elevated CRP when compared to 71(75.53%) mild to moderate cases. CRP level was higher during

early stage of severe Covid-19 disease (0-7 days of hospital admission). The better accuracy of biomarker CRP in predicting severe illness when compared to mild-moderate disease have been reported. However, the present study showed no significant correlation between elevated CRP marker and severity of Covid-19. Similarly, no positive correlation was found between CRP and RTPCR positive and negative Covid cases. CRP is reliable and sensitive biomarker in reflecting Covid-19 disease development [19,20]. Prior to CT scan critical findings, C reactive protein may be significantly increased in the initial phase of severe COVID-19 infection. In a similar study report by Tan et al., CRP values were found to be more reliable for the early identification of case severity when compared with CT scans [21].

 Covid-19 disease severity leads to tissue damage and subsequent release of larger quantity of Lactate Dehydrogenase enzyme into blood circulation indicating a severe interstitial pneumonia [22]. In our study 12(37.5%) severe Covid cases had elevated LDH. No statistical significance was observed with respect to the correlation between biomarker, LDH and patient's RTPCR assay result as well as LDH and mild, moderate and severe Covid. Xiong et al have reported the positive correlation between LDH level and CT scan to evaluate severe Covid-19 pneumonia [23].

Increased levels of D –dimer were invariably reported in Covid patients with prevalence ranging from 43-68% [24]. In our study 10(50.00%) severe Covid cases had elevated levels of D dimer. There was no significant correlation observed among patients with increased D Dimer level and severity of disease in our study. However significant correlation between levels of D dimer and severity and D dimer assay as an early biomarker of severity even before Chest CT scan or as complement to Chest CT scan and clinical staging have been reported [17]. D dimer levels on admission could be used for sorting of patients into intensive care facility [25]. The

predictive accuracy of D -Dimer and serum Ferritin in identifying cases with pneumonia has been reported [20].

Elevated serum ferritin level due to cytokine storm have been described in severe Covid. Twenty (25.97%) severe Covid cases in our study had increased levels of serum Ferritin. There was no significant correlation observed in cases with elevated serum Ferritin and disease severity in our study. In a study report, high mortality rate was observed among patients with Ferritin levels >1000 and 89% patients with Ferritin levels >1000 were reported to have pneumonia [18].

Laboratory biomarkers may play an important role in assessing disease staging based on severity of illness, patient admission protocol, patient monitoring and management. However no single biomarker assay was found to have a noteworthy role in diagnosis or to exclude severe Covid cases. Effective biomarkers would be essential for Covid-19 screening, clinical management and for the prevention of Covid related complication.

Limitation of the study

We have investigated the potential role of CRP, LDH, Ferritin and D dimer in Covid disease severity. Other biochemical parameters and haematological abnormalities in Covid patients were not assessed. Patients with no lung involvement observed with CT scan were excluded from the study. The retrospective nature of the data and less sample size makes the present study lacks generalizability.

CONCLUSION

More than 80% Covid cases showed elevated biomarker C- reactive protein. Increased LDH level was observed among 28.32% patients. Elevated D dimer was observed among 17.70% cases. More than 60% cases showed elevated serum Ferritin. In our study no positive correlation was observed between serum markers

and Covid-19 disease severity. No significant association observed between study groups, disease severity and serum biomarkers. The role of multiple serum biomarker in the initial stage of Covid diagnosis of clinically suspected cases need to be further assessed.

Source of Funding: Nil

Conflicts of Interest: Nil

REFERENCES

1.Guo L, Bi W, Wang X, Xu W, Yan R, Zhang Y,et al. Engineered trimeric ACE2 binds viral spike protein and locks it in "Three-up" conformation to potently inhibit SARS-CoV-2 infection. *Cell Research.* 2021;31(1):98–100.https://doi.org/10.1038/s41422-020-00438-w

2.Letarov AV, Babenko VV, Kulikov EE. Free SARS-CoV-2 spike protein S1 particles may play a role in the pathogenesis of COVID-19 infection. *Biochemistry* (Mosc).2021;86(3):257–61.doi: 10.1134/S0006297921030032

3. Guan W, Ni Z, Hu Y, Liang W, Ou C, He J et al. Clinical characteristics of coronavirus disease 2019 in China. *New England Journal of Medicine* 2020; 382:1708-1720
DOI: 10.1056/NEJMoa2002032

4. Tang N, Li D, Wang X, Sun Z Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *Journal of Thrombosis and Haemostasis*,2020 ;18(4):844–84DOI: 10.1111/jth.14768

5. Battaglini D, Lopes-Pacheco M, Castro-Faria-Neto HC, Pelosi P, Rocco PRM. Laboratory Biomarkers for Diagnosis and Prognosis in COVID-19. *Frontiers in Immunol*ogy 2022;27:13:857573. doi: 10.3389/fimmu.2022.857573. .

6.Diao B, Wang C, Tan Y, Chen X, Liu Y, Ning L et al . Reduction and functional exhaustion of T cells in patients with coronavirus disease 2019 (COVID-19). *Frontiers in Immunology* 2020;11:827DOI: 10.3389/fimmu.2020.00827

7. Chalmers S, Khawaja A, Wieruszewski PM, Gajic O, Odeyemi Y. Diagnosis and treatment of acute pulmonary inflammation in critically ill patients: the role of inflammatory biomarkers. *World Journal of Critical Care Medicine* 2019;8(5):74–96doi: 10.5492/wjccm.v8.i5.59

8.Vargas-Vargas M, Cortés-Rojo C. Ferritin levels and COVID-19. *Rev Panama Salud Publication*. 2020;44:e72 https://doi.org/10.26633/RPSP.2020.72

9 .Han Y, Zhang H, Mu S. Lactate dehydrogenase, a risk factor of severe COVID-19 patients. medRxiv 2020.**doi:** https://doi.org/10.1101/2020.03.24.20040162

10.Scott Gutovitz, Justin Hanson, Christian Vandever, Dietrich Jehle.Clinical Characteristics and Laboratory Biomarkers for Patients with Suspected COVID-19 Infection Within HCA Healthcare.HCA Healthcare Journal of Medicine 2021; 2(3):229-236.DOI: 10.36518/2689-0216.1233

11.Wang D,HuB,HuC,ZhuF,Liu X ,Zhang J et al.Clinical Characteristics of 138 hospitalised patients with 2019 novel Corona virus-infected pneumonia in Wuhan,China JAMA 2020;323(11):1061-1069. doi:10.1001/jama.2020.1585 Erratum in: *Journal of American Medical Association.* 2021 Mar 16;325(11):1113.

12. Lingaiah A, Srinivasarao Bandaru. VCS Role of Biomarker in COVID-19: A Study from Tertiary Care Centre. *Annals of Infectious Disease and Epidemiology*,2021; 6(1): 1062. ISSN: 2475-5664

13. Das B, Bhatia S Y,Pal PM. Evaluation of the role of Routine Laboratory biomarkers in Covid 19 patients: Prospective from a tertiary care hospital. *Indian Journal of Clinical Biochemistry,* 2021;36(4):473-484.doi: 10.1007/s12291-021-00978-x

14.Chen L, Liu HG, Liu W, Liu J, Liu K, Shang J et al. Analysis of clinical features of 29 patients with 2019 novel coronavirus pneumonia. *Zhonghua Jie He He Hu Xi Za Zhi.* 2020 ; 6:43(0):E005. Chinese. doi: 10.3760/cma.j.issn.1001-0939.2020.0005.

15.Liu Y, Yang Y, Zhang C, Huang F, Wang F, Yuan J, et al.Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Science China Life Sciences.* 2020 ;63(3):364-374. doi: 10.1007/s11427-020-1643-8

16.Poudel A, Poudel Y, Adhikari A, Aryal BB, Dangol D, Bajracharya T, et al. D-dimeras a biomarkerfor assessment of COVID-19prognosis: D-dimer levels on admission and its role in predicting disease outcome in hospitalized patients with COVID-19.*PLoS ONE*,2021; 16(8): e0256744. https://doi.org/10.1371/journal.pone.0256744

17.Yao Y, Cao J, Wang Q, Shi Q, Liu K, Luo Z et al .D-dimer as a biomarker for disease severity and mortality in COVID-19 patients: a case control study.*Journal of Intensive Care* 2020;. 10;8:49. doi: 10.1186/s40560-020-00466-z

18. Alroomi M, Rajan R, Omar AA, Alsaber A, Pan J, Fatemi M et al .Ferritin level:A predictor of severity and mortalityin hospitalized COVID-19patients.*Immunity Inflammation and Disease*. 2021;9:1648–1655 DOI: 10.1002/iid3.517

19. Kermali M, Khalsa RK, Pillai K, Ismail Z, Harky A. The role of biomarkers in diagnosis of COVID-19–A systematic review. *Life sciences.* 2020,1;254:117788.. doi: 10.1016/j.lfs.2020.117788.

20.Farid E, Sridharan K, Alsegai OA, Khawaja SA, Mansoor EJ, Teraifi NA.Utility of inflammatory biomarkers in patients with COVID-19 infections: Bahrain experience. *Biomarkers in, Medicine.*2021;15(8),541–549.DOI: 10.2217/bmm-2020-0422

21.Tan C, Huang Y, Shi F, Tan K, Ma Q, Chen Y,.C reactive protein correlates with computed tomographic findings and predicts severe Covid 19 early .*Journal of Medical Virology* 2020. ;92(7):856-862. doi:10.1002/jmv.25871.

22.Wu M Y, Yao L, Wang Y, Zhy X Y, Wang X F,Tang P J, et al. Clinical evaluation of potential usefulness of serum lactate dehydrogenase(LDH) in 2019 novel Corona Virus (COVID-19) pneumonia. *Respirarory Research* 2020;21(17).https://doi.org/10.1186/s12931-020-01427-8

23.Y Xiong,D Sun,Y Liu,Y Fan,I Zhao,X Li et al.Clinical and high resolution CT features of Covid -19 infection.Comparison of the initial and followupchanges.*Investigative Radiolology* 2020;55(6)332-339.

24.Li X, Xu S, Yu M, Wang K, Tao Y, Zhou Y.Risk factors for severity and mortality in adult covid 19 patients in Wuhan. *Journal of Allergy and Clinical Immunolology* 2020.;146(1):110-118. doi: 10.1016/j.jaci.2020.04.006

25.C Huang,Y Wang,X Li,Li Ren,J Zhao,Y Hu .Clinical features of patients infected with 2019 novel coronavirus in Wuhan China Lancet 395(10233) 2020 :497-506https://doi.org/10.1016/S0140-6736(20)30183-5