Vasculitis-Associated Auto-antibodies and Complement Levels in patients with COVID-19 Infection

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Research Article

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Abstract

Introduction / objectives: The cause of coronavirus disease 2019 (COVID-19) is severe acute respiratory syndrome 2 (SARS-CoV-2). There are evidences of involvement of immune system in pathogenesis of this disease. We investigated the presence of various vasculitis-associated auto-antibodies and complement levels in a series of patients with COVID-19 infection admitted to our hospital.

Methods: Forty patients with severe or critical type of COVID 19 were evaluated for symptoms, signs and laboratory tests of vasculitis syndromes including rheumatoid factor (RF), anti-nuclear antibody (ANA), anti dsDNA, c and p anti-neutrophilic cytoplasmic antibody (c ANCA and p ANCA) and complement levels. Descriptive statistics methods were used to describe the clinical / laboratory findings.

Results: Forty patients with severe to critical illness were enrolled in the study. The mean age of the patients was 48.5 ± 9.8 years. All patients had pulmonary involvement in lung CT scan. Lymphopenia in 19 (47.5%), raised creatinine in 8(20%) and hyperbilirubinemia in 19(47.5%) of patients were seen. Vasculitis laboratory test results included: RF in 2 patients, ANA in 3 patients and ANCA in one patient. 17(42.5%) of patients had hypocomplementemia in one or more complement tests. Of the four patients who were expired, three had a decrease in complement.

Conclusion: In 17 of patients (42.5%) we detected low complement levels. A decrease in complement levels may predict a critical state of the disease. Therefore, measuring its levels may be helpful in making earlier decisions to initiate disease-suppressing treatments, including corticosteroids and IVIG.

Key Points

- Pathogenesis of severe disease forms and complications of COVID–19 is unclear.
- Detection of biomarkers for early diagnosis of severe and critical cases may be helpful in better stratification and proper management of the disease.
- Hypocomplementemia may predict critical and/or severe cases of COVID–19.

Introduction

Coronavirus disease 2019 (COVID–19) is caused by severe acute respiratory syndrome coronavirus and the first cases were classified as “pneumonia of unknown etiology.” The disease represents a potentially fatal disease of great global public health importance. As of May 4, 2020, the outbreak of COVID–19 has resulted in 3,610,189 confirmed cases and 250,099 deaths globally.[1–3] Symptoms of the disease can occur in different systems. Fever, cough, pulmonary and cardiac complications, headache, lymphopenia, increased lactic dehydrogenase, coagulation disorders, increased liver and muscle enzymes, and electrolyte disturbances are clinical and or laboratory findings of the disease.[2, 4–8] Severe COVID–19 can lead to acute respiratory distress syndrome and multiple organ dysfunction, which is the leading causes of death.[9] Infection has been associated with numerous direct and indirect cardiovascular

complications, including acute myocardial infarction, myocarditis, arrhythmias, and venous thromboembolism.[5] Information about kidney involvement as a pathological diagnosis is relatively incomplete and limited.[10]

Due to the lack of specific antiviral drugs, current treatment of the disease is mainly supportive.[2, 4] However, there are currently several therapies being used to treat this life-threatening condition.[11–13]. Increasing knowledge about the pathophysiology of SARS-CoV–2 infection has led to some anti-rheumatic drugs being considered as possible treatment options for COVID–19 treatment.[14–16] Studying the relationship between vasculitis syndromes or related laboratory findings with COVID 19 may be interesting in several ways:1. Given the variety of symptoms in both diseases, is there a similarity in the mechanism of injury? 2. Understanding the pathways of damage in any disease may be of great help in identifying its treatment.3. Can the treatments used for the infection modify or ignite the pathophysiological processes of the disease? Designing and conducting this study can suggest ways to identify these changes, the safety pathways involved, and possibly the use of appropriate treatments. The aim of this study was to investigate seropositivity of the tests used for the diagnosis of vasculitis syndromes in patients with severe to critical COVID 19 infection.

Patients And Methods

Study population: The study was a cross sectional study conducted on April 2020 in hospitalized patients with COVID 19 attending in a referral public hospital in Sari, North of Iran. A rheumatologist consecutively screened patients for possible inclusion. Due to the lack of previous studies in this field, the study was performed on 40 eligible patients.

Selection criteria: Positive diagnosis of COVID–19 was based on compatible clinical symptoms with positive PCR and/ or positive findings in lung CT scan in accordance with infection.[17] Definition of disease severity: Based on the symptoms of the disease, it was classified into three categories: mild, severe and critical; mild: no pneumonia or mild pneumonia, severe: shortness of breath, respiratory rate ≥ 30 per minute, oxygen saturation ≤93%, pulmonary infiltration more than 50% within the first 24–48 hours, and critical: respiratory failure, septic shock, dysfunction or multiple organ failure. [18] Patients with severe or critical manifestations were included in the study.

Inclusion and exclusion criteria:

Patients in the age range of 18–6o years old having either severe or critical form of the COVID–19 were enrolled. On the other hand patients having a positive history of malignancies, chronic liver or kidney diseases, history of rheumatic inflammatory diseases (including systemic lupus, rheumatoid arthritis, scleroderma or vasculitis associated with anti-neutrophil cytoplasmic antibodies or anti-nuclear antibodies) or using a moderate or high dose of steroids (prednisolone more than 20 mg daily) or immunosuppressive and biological drugs were excluded.

Ethics approval: This study was approved by the Vice Chancellor for Research and Technology and Ethics Community of Mazandaran University of Medical Sciences (Ethic code: IR. MAZUMS. REC.1399.108). Patients or their companions were explained about the study.

Data collection: After selecting eligible patients their consent was collected, clinical and demographic information of patients was recorded. Laboratory data including CBC, biochemistry, coagulation tests, urine analysis and reports of the lung CT scans were recorded. Ten ml of blood was drawn from patients and the sera were stored at 2–8 C.

Vasculitis syndrome tests include rheumatoid factor (RF) tests, antinuclear antibodies (ANA), anti-double stranded DNA (anti dsDNA), anti-neutrophil cytoplasmic antibodies (ANCA), and measurement of complement levels (C3, C4 and C50). RF was estimated by Latex method (Bionic, Iran) and ANA, anti dsDNA antibodies and ANCAs were performed by ELISA (EUROIMMUN, Germany) and a value greater than the upper limit of normal was considered positive. The levels of complement were also measured by ELISA (Pars Azmoon, Iran and Diametra, China) and values less than the lower limit of normal were considered low.

Leukopenia was defined as WBC < 4400 cells/microL and lymphopenia was considered if absolute count was <1000 cells/microL. Anemia was defined as Hgb<12g/dl in women and <13.5 in men. Thrombocytopenia was considered as plt<150000/ microL. High serum creatinine in men and women were considered as 1.13 and 0.93 mg/dl consequently. Elevated alanine transferase (ALT) considered if it was more than 55 IU/L in male or 32 IU/L in female. Hyperbilirubinemia was defined as total bilirubin higher than 1 mg/dl, and direct bilirubin more than 0.4 mg/dl. CPK and troponin were described high if levels more than 195 in men and 170 in women for CPK U/L and higher than 100 ng/dl for troponin.

Statistical analysis: Descriptive statistics methods such as mean and percentage were used to describe the clinical / laboratory findings and Chi-square to compare qualitative variables.

Results

Forty patients with severe to critical illness entered the study. The mean age of the patients was 48.5 ± 9.8 years and twenty-seven patients (67.5%) were male. The mean duration of symptoms was 14.0 ± 5.9 days.Demographic and basic clinical data of patients with COVID 19 involvement are shown in table 1.

The selected patients were categorized as severe or critical and patients with mild manifestations were not included the study. All patients had significant changes of COVID 19 pneumonia in their CT scans and also clinical manifestations of COVID 19 infection. The mean± SD of respiratory rate and saturation pressure of O2 were 23.17±5.8/min and 87.15% ± 7.46.

All patients received hydroxychloroquine and lopinavir/ritonavir combination therapy, in addition they were treated by interferon beta–1 in 27 patients, parenteral glucocorticoid in 17 of cases, IVIG in 8

patients, umifenavir) in 6 patients, daclatasvir/sofosbuvir) in 4 cases, ribavirin in 3 patients and tocilizumab and remdesivir in one patient.

The vasculitis tests results are shown in table 2. Of the 40 patients, seventeen (42.5%) had hypocomplementemia in one or more components (low C3 in 9 patients, low C4 in 5 patients and low CH50 in 14 of them). Six of the 15patients with severe disease (40%) and eleven of the 25 critically ill patients (44%) had low complement (P = 0.804). Regarding lung involvement, the number of lobes involved, or the percentage of involvement, there was no difference between the two groups with low and normal complement (P>0.05). Four patients expired, three of whom had a significant reduction in complement.

Table 1. Demographic and basic clinical data of patients with COVID 19 involvement.

Variables category			Additional description
Demographic data	Age (years, mean± SD)	48.50 ± 9.84	
	Male sex (number, %)	27 (67.5%)	
	Urban residency (number, %)	27 (67.5%)	
Underlying diseases	Diabetes mellitus (number, %)	11 (27.5%)	One patient was pregnant
	Hypertension (number, %)	5 (12.5%)	
	Ischemic heart disease (number, %)	6 (15%)	
	Hyperlipidemia (number, %)	1 (2.5%)	
	Asthma or COPD (number, %)	3 (7.5%)	
COVID 19 disease manifestation	Disease duration (days, mean± SD)	14.07 ± 5.94	One patient had pericardial effusion. None of the patients had skin rash or arthritis.
	Severe vs critical illness (number, %)	15 (37.5%)/ 25 (62.5%)	
	Fever or chills (number, %)	22 (55%)	
	Myalgia (number, %)	20 (50%)	
	Headache (number, %)	5 (12.5%)	
	Loss of consciousness (number, %)	6 (15%)	
	Dyspnea (number, %)	28 (70%)	
	Cough (number, %)	22 (55%)	
	Nausea and/or vomiting	8 (20%)	

	(number, %)		
	Diarrhea (number, %)	2 (5%)	
	Abdominal pain (number, %)	2 (5%)	
	Chest pain (number, %)	3 (7.5%)	

Table 2. Laboratory and imaging finding in patients with COVID 19 involvement.

Variables category			
Laboratory findings	CBC	Lymphopenia (number, %)	19 (47.5%)
		Anemia (number, %)	27 (67.5%)
		Thrombocytopenia (number, %)	8 (20%)
	Acute phase reactants	ESR, mm/h (mean± SD)	52.42±22.92
		CRP, mg/dl (mean± SD)	53.34±52.19
	Blood chemistries	Elevated ALT (number, %)	6 (15%)
		Elevated CPK (number, %)	15 (37.5%)
		Elevated troponin (number, %)	2 (5%)
		Raised creatinine (number, %)	8 (20%)
		Hyperbilirubinemia, direct (number, %)	19 (47.5%), 18 (45%)
	Coagulation tests study	Prolonged PT (number, %)	11 (27.5%)
		Prolonged PTT	2 (5%)
	Urine analysis	Abnormal UA (number, %)	7 (17.5%)
	Vasculitis lab tests	Positive RF (number, %)	2 (5%)
		Positive ANA (number, %)	3 (7.5%)
		Positive Anti dsDNA (number, %)	None
		Low complement levels (number, %)	17(42.5%)
		Positive C ANCA (number, %)	1(2.5%)
		Positive P ANCA (number, %)	
Lung CT scan findings	Number of involved lobes (mean± SD)		4.08 ± 0.87
	Percentage of lung involvement (mean± SD)		56.62%± 14.77
	Peripheral opacity (number, %)		8 (20%)
	Grand glass opacity (number, %)		34 (85%)
	Consolidation (number, %)		16 (40%)

Discussion

In the present study, we examined vasculitis tests in patients with COVID 19. Three patients had a positive ANA result, of which two had a hypocomplementemia and they were also being treated with interferon. All

three patients were discharged from the hospital with full recovery, despite severe pulmonary involvement initially. One patient was positive both for C and P ANCA, with pancytopenia but without any other ANCA associated vasculitis manifestations. Seventeen of the patients, showed a decrease in one or more components of the complement and of the four patients who were expired, three had a decrease in complement.

The vasculitides are defined by the presence of inflammatory leukocytes in the vessel walls with damage to mural structures. This damage may lead to bleeding due to loss of vessel integrity or tissue ischemia and necrosis due to compromise of the lumen. They are diagnosed based on patterns of organ injury, the size of the vessels affected, histopathological and imaging features. ANA, ANCA and serum complement levels are laboratory tests that help the physician in knowing the pathophysiology and diagnosis.[19] The complement system is a major component of innate immunity and an effector arm of humoral immune system. It also serves as a mechanism to identify and clear injured tissue and cellular debris. Although the complement system is primarily perceived as a host defense system but it may be a potentially more harmful side of innate immune pathway as an inflammatory mediator. [20] Severe sepsis is an acute condition that leads to activation of the complement and coagulation cascades and may play central roles in multiple organ failure and severe complications.[21] Intrapulmonary activation of complement can cause acute lung injury that is complement and PMN-dependent, resulting in a cytokine storm.[22] There are some studies identify the role of complement system as an important host mediator of SARS-CoV-induced disease and suggest that complement activation regulates a systemic proinflammatory response to SARS-CoV infection. It was investigated that inhibition of complement signaling might be an effective treatment option following SARS-COV infection by using a mouse model. [23] Hypocomplementemia is an early diagnostic marker of parvovirus B19 infection in adult.[24] Activation of the complement pathways have been reported in chronic hepatitis C virus (HCV) infection and there is an association between HCV pathogenesis and abnormal complement profiles.[25] Creating an immune complex and activating the complement system is one of the basic mechanisms in the pathophysiology of many autoimmune and vasculitis syndromes.

A major concern in the treatment of critically ill patients is drug side effects. There are a broad spectrum of interferon-related complications including glomerulonephritis, systemic lupus erythematosus-like syndrome and thrombotic microangiopathy that may be associated with hypocomplementemia. [26, 27] Drug-induced lupus erythematosus (DILE) is a syndrome with clinical and serological features similar to systemic lupus erythematosus (SLE) that is temporally related to continuous drug exposure and which resolves after discontinuation of this drug. More than 90 drugs, including interferons, are involved in causing the disease.[27] In this study, 15 of the 27 patients receiving interferon showed a deficiency of complement. Low levels of complement in these patients may be due to disease or interferon use.[28] Therefore, in addition to proper and appropriate use of the drug, care should be taken in selecting patients and monitoring its side effects.

In this study, we did not find any classic vasculitis syndrome, but the variety of symptoms in patients with COVID–19 can be similar and mimic the syndromes of vasculitis. We entered severe or critical cases of

the disease only. Comparing these cases with mild cases of the disease may reveal more findings related to hypocomplementemia. Besides that, many patients were being treated with various medications that may affect the results.

We encountered limitations in the study. First: this study was performed as a pilot on these patients and according our knowledge, evaluation of vasculitis tests and complement levels in COVID–19 have not yet been published. Therefore, we did not have any previous study to estimate the number of samples based on them. Second: patients were examined after receiving certain medications, such as interferon or steroids, subsequent studies can be performed before treatment interventions or on different days of treatment.

Conclusion

Based on the results of the present study, a decrease in complement may predict a critical state of the disease. Therefore, measuring its levels may be helpful in making earlier decisions to initiate disease-suppressing treatments, including corticosteroids and IVIG. This was a small, pilot study and for a definite conclusion, more studies with larger sample size and inclusion of milder forms of the disease as a control group are needed.

Declarations

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Conflict of interest statement

The authors declare no conflict of interest with respect to the research, authorship or publication of this article.

References

1. Tu H, Tu S, Gao S, Shao A, Sheng J (2020) The epidemiological and clinical features of COVID-19 and lessons from this global infectious public health event. The Journal of infection.
2. Cascella M, Rajnik M, Cuomo A, Dulebohn SC, Di Napoli R (2020) Features, Evaluation and Treatment Coronavirus (COVID-19). Stat Pearls Publishing.
3. *https://www.worldometers.info/.coronavirus/.*
4. Goh KJ, Choong MC, Cheong EH, Kalimuddin S, Duu Wen S, Phua GC, et al (2020) Rapid Progression to Acute Respiratory Distress Syndrome: Review of Current Understanding of Critical Illness from COVID-19 Infection. Annals of the Academy of Medicine, Singapore. 49(1):1-9.

5. Driggin E, Madhavan MV, Bikdeli B, Chuich T, Laracy J, Bondi-Zoccai G, et al (2020) Cardiovascular Considerations for Patients, Health Care Workers, and Health Systems During the Coronavirus Disease 2019 (COVID-19) Pandemic. Journal of the American College of Cardiology.
6. Wan S, Xiang Y, Fang W, Zheng Y, Li B, Hu Y, et al (2020) Clinical Features and Treatment of COVID-19 Patients in Northeast Chongqing. Journal of medical virology.
7. Zhang X, Cai H, Hu J, Lian J, Gu J, Zhang S, et al (2020)Epidemiological, clinical characteristics of cases of SARS-CoV-2 infection with abnormal imaging findings. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases.
8. Omrani-nava V, Maleki I, Ahmadi A, Moosazadeh M, hedayatzadeh-omran A, Roozbeh F, et al (2020) Evaluation of hepatitis enzymes changes and association with prognosis in COVID-19 patients. Hepat Mon. 20(4): e103179.
9. Li HC, Ma J, Zhang H, Cheng Y, Wang X, Hu ZW, et al (2020) Thoughts and practice on the treatment of severe and critical new coronavirus pneumonia. Zhonghua jie he he hu xi za zhi = Zhonghua jiehe he huxi zazhi = Chinese journal of tuberculosis and respiratory diseases. 43(0):E038.
10. Wang HJ, Du SH, Yue X, Chen CX (2020) Review and Prospect of Pathological Features of Corona Virus Disease. Fa yi xue za zhi. 36(1):16-20.
11. Bhatnagar T, Murhekar MV, Soneja M, Gupta N, Giri S, Wig N, et al (2020) Lopinavir/ritonavir combination therapy amongst symptomatic coronavirus disease 2019 patients in India: Protocol for restricted public health emergency use. The Indian journal of medical research.
12. Dhama K, Sharun K, Tiwari R, Dadar M, Malik YS, Singh KP, et al (2020) COVID-19, an emerging coronavirus infection: advances and prospects in designing and developing vaccines, immunotherapeutics, and therapeutics. Human vaccines & immunotherapeutics. 1-7.
13. Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M, et al (2020) Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. International journal of antimicrobial agents. 105949.
14. Favalli EG, Ingegnoli F, De Lucia O, Cincinelli G, Cimaz R, Caporali R(2020)COVID-19 infection and rheumatoid arthritis: Faraway, so close! Autoimmunity reviews. 102523.
15. Gao J, Tian Z, Yang X (2020) Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. Bioscience trends. 14(1):72-3.
16. Tanaka T (2015) IL-6 blockade therapy for inflammatory diseases: current perspectives and future directions. Nihon Rinsho Men'eki Gakkai kaishi = Japanese journal of clinical immunology. 38(6):433-42.
17. Liu KC, Xu P, Lv WF, Qiu XH, Yao JL, Gu JF, et al (2020) CT manifestations of coronavirus disease-2019: A retrospective analysis of 73 cases by disease severity. European journal of radiology. 126:108941.
18. Wu Z, McGoogan JM (2020) Characteristics of and Important Lessons from the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases from the Chinese Center for Disease Control and Prevention. Jama.

19. Morita T, Tres GFS, Criado RFJ, Sotto MN, Criado PR (2016) Update on vasculitis: an overview and dermatological clues for clinical and histopathological diagnosis - part I. Anais brasileiros de dermatologia. 2020.
20. Ricklin D, Reis ES, Lambris JD (2016) Complement in disease: a defence system turning offensive. Nature reviews Nephrology. 12(7):383-401.
21. Holers VM (2014) Complement and its receptors: new insights into human disease. Annual review of immunology. 32:433-59.
22. Bosmann M, Ward PA (2012) Role of C3, C5 and anaphylatoxin receptors in acute lung injury and in sepsis. Advances in experimental medicine and biology. 946:147-59.
23. Lisa E. Gralinski, Timothy P. Sheahan, Thomas E. Morrison, Vineet D. Menachery, Kara Jensen, Sarah R. Leist, et al (2018)Complement Activation Contributes to Severe Acute Respiratory Syndrome Coronavirus Pathogenesis. mBio. 9(5):e01753-18.
24. Hashizume H, Kageyama R (2017)Hypocomplementemia is a diagnostic clue for parvovirus B19 infection in adults. The Journal of dermatology. 44(3):e27.
25. El-Shamy A, Branch AD, Schiano TD, Gorevic PD (2018) The Complement System and C1q in Chronic Hepatitis C Virus Infection and Mixed Cryoglobulinemia. Frontiers in immunology.9:1001.
26. Gianassi I, Allinovi M, Caroti L, Cirami LC (2019) Broad spectrum of interferon-related nephropathies-glomerulonephritis, systemic lupus erythematosus-like syndrome and thrombotic microangiopathy: A case report and review of literature. World journal of nephrology. 8(7):109-17.
27. Araujo-Fernandez S, Ahijon-Lana M, Isenberg DA(2014) Drug-induced lupus: Including anti-tumour necrosis factor and interferon induced. Lupus. 23(6):545-53.
28. Vedove CD, Del Giglio M, Schena D, Girolomoni G (2009) Drug-induced lupus erythematosus. Archives of dermatological research. 301(1):99-105.