Positive Epstein-Barr virus detection in corona virus disease 2019 (COVID-19) patients

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Abstract

Background Since December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused an outbreak of corona virus disease 2019 (COVID-19) in Wuhan, China. The Objective of this study was to detect the EBV coinfection in COVID-19.

Methods In this retrospective single-center study, we included 67 patients with confirmed COVID-19 in Renmin Hospital of Wuhan University from January 9 to February 29, 2020. Patients were divided into EBV seropositive group and seronegative group according to the serological results of EBV, and the characteristics differences between the two groups were compared.

Results 67 COVID-19 patients were included in our study. The median age was 37 years, with 35 (52.2%) females. Among these COVID-19 patients, 37 (55.2%) patients were seropositive for EBV viral capsid antigen (VCA) IgM antibody. EBV seropositive COVID-19 patients had a 3.09-fold risk of having a fever symptom than EBV seronegative (95%CI, 1.11-8.56; P=0.03). C-reactive protein (CRP) (P=0.02) and the aspartate aminotransferase (AST) (P=0.04) in EBV seropositive COVID-19 patients were higher than that in EBV seronegative patients. EB seropositive patients had a higher portion of corticosteroid use than the EB seronegative patients (P=0.03).

Conclusions EBV acute infection was found in COVID-19 patients. EBV seropositivity was associated with fever and increased inflammation. EBV reactivation may affected the treatment of COVID-19.

Introduction

Since December 2019, a novel coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused an outbreak of Coronavirus Disease 2019 (COVID-19) in Wuhan, China[1, 2]. SARS-CoV-2 was highly contagious and has rapidly spread. According to the date of the World Health Organization (WHO), as of 10:00 CET April 5, 2020, the total number of confirmed cases reached 1,133,758 globally, and more than 200 countries and regions have been reported confirmed cases[3]. COVID-19 developed rapidly for critically ill patients, according to the report, the median time from symptom onset to ICU admission was 9.5 days, and the median time from ICU admission to death was 7 days [4]. Unfortunately, there was no proven effective treatment for coronavirus except for supportive care [5].

According to the laboratory results of COVID-19 patients, it was noted that some patients were positive for Epstein-Barr virus (EBV) viral capsid antigen (VCA) IgM antibody. Although the infection rate of EBV is up to 90% in the adult population, most immunocompetent people have no clinical manifestations after infection [6]. However, it can be reactivated and proliferated in immunocompromised individuals, with fatal outcome[7]. EBV infection have been reported in some carcinomas such as Burkitt lymphoma, nasopharyngeal carcinomas and T-cell/NK lymphoma, as well as autoimmune diseases including systemic lupus erythematosus (SLE) and multiple sclerosis (MS) [8–10]. Recently, the pathological report of COVID-19 dead patient suggested that there was overactivation of T cells, which to some extent led to

severe immune injury in COVID-19 patients [11]. Moreover, there are similar symptoms such as fever, fatigue, myalgia, anorexia and sore throat between COVID-19 [12, 13] and EBV-induced infectious mononucleosis (IM), indicating a potential association.

We hypothesized that EBV positivity may have an effect on the clinical characteristics, immune response and the outcome in COVID-19 patients. The aim of this study was to describe the clinical characteristics of patients with confirmed SARS-CoV-2 infection, and compare the differences between EBV seropositive and seronegative COVID-19 patients, so as to find out whether EBV positivity affects the disease progression and give a clue to clinical judgment and treatment.

Methods

Study population

COVID-19 hospitalized patients were enrolled from January 9 to February 29, 2020 at Renmin Hospital of Wuhan University in Wuhan, Hubei province, China. The inclusion criteria in our study were as follows: (1) At least one positive result by real-time quantitative reverse-transcriptase-polymerase-chain reaction (RT-PCR) assay for SARS-CoV-2 when in hospital; (2) Measuring the antibodies against EBV VCA (IgM, IgG), EBV early antigen (EA, IgM) and EBV nuclear antigen (EBNA, IgG); (3) Time of the onset of symptoms to hospital admission less than 2 weeks. Exclusion criteria: (1) In hospital time later than February 29, 2020; (2) Most clinical information were missing. The discharge criteria in our study was according to the diagnosis and treatment protocol for COVID-19 from the National Health Commission of the People's Republic of China[14]: 1 Afebrile for more than 3 days; 2 Respiratory symptoms significantly improved; 3 Obvious improvement in the radiological abnormalities on chest radiograph; 4 Two consecutive negative SARS-CoV-2 nucleic acid tests at least 24 h intervals. The clinical outcomes of our COVID-19 patients were followed up to March 16, 2020. The recovery time was defined as the time from the onset of symptoms to the time of discharge. The study was approved by the ethical committee board of Renmin Hospital of Wuhan University (WDRY2020-K073). Written informed consent was waived due to the rapid emergence of this infection disease.

Data Collection

The clinical information about the demographic characteristics (ie, age, sex, comorbidities), symptoms, signs, laboratory tests and CT results, treatments and clinical outcomes (discharge or inpatient) were obtained from the electronic medical records. Two researchers (TC and HLL) recorded the data independently and any differences were resolved by checking the original records. The durations from onset of the first symptom to hospitalization was also recorded. The laboratory tests include the standard blood counts (ie, white blood cell count, lymphopenia count), blood biochemistry (ie, alanine aminotransferase, aspartate aminotransferase, Prealbumin), C-reactive protein (CRP), humoral immunity (ie, IgG, IgM, C3 and C4) and cellular immunity (ie, CD3 count, CD4 count and CD8 count).

Sample Collection And Pathogens Detection

Antibodies against EBV VCA (IgM, IgG), EA IgM and EBNA IgG were detected by Chemiluminesent Immunoassay Assay (CLIA). The EBV seropositive was defined as VCA IgM positive and EBV seronegative was defined as VCA IgM negative. Nasopharyngeal swabs were collected from all patients to test for SARS-CoV-2 by real-time RT-PCR according to the same protocol described previously[15]. Other 13 respiratory viruses including the influenza A virus (IFV-A), H1N1, H3N2, influenza B virus (IFV-B), parainfluenza virus (PIV), respiratory syncytial virus (RSV), human metapneumovirus, SARS-CoV, rhinovirus, adenovirus (ADV), Bocavirus, mycoplasma pneumonia and chlamydia were also detected by polymerase chain reaction (PCR) Capillary Electrophoresis Fragment Analysis. Indirect immunofluorescence (IIFA) was used to examine the specific IgM of 9 respiratory pathogens. These pathogens were *legionella pneumophila* (LP), *mycoplasma pneumonia* (MP), *Q fever pneumonia* (COX), *chlamydia pneumoniae* (CP), ADV, RSV, IFV-A, IFV-B and PIV. The antibodies against cytomegalovirus (CMV, IgM and IgG) were also tested.

Statistical Analysis

Frequency variables were reported as numbers and percentages and compared by χ^2 test or Fisher's exact. Continuous data were described as median (interquartile range [IQR]), and compared with t test or the Wilcoxon test. The analysis comparing between the EBV seropositive and seronegative COVID-19 patients were performed. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. Statistical analysis was performed with SAS software (SAS 9.3; SAS Institute Inc, Cary, North Carolina, USA). All P values were two-sided and the statistically significant value was < 0.05.

Results

Patients Characteristics

Figure 1 showed the workflow of COVID-19 patients' inclusion and exclusion criteria. Among 210 hospitalized patients from January 9 to February 29, 2020, 188 patients had positive SRAS-CoV-2 RT-PCR results. According to the inclusion and exclusion criterion, 67 COVID-19 patients with the results of anti-EBV antibodies were included to the final analysis. The median age of these COVID-19 patients was 37 years (IQR, 30–52; range, 23–81 years), with 35 (52.2%) were females. The median durations from onset of the first symptom to hospitalization was 4 days (IQR: 2–7 days) (Table 1). Among the 67 COVID-19 patients, 11 (16.4%) had 1 or more combined diseases, as follows, cardiovascular disease (4 [6.0%]), hypertension (4 [6.0%]), diabetes (3 [4.5%]) and chronic liver disease (2 [3.0%]) and digestive system disease (1 [1.5%]).

Table 1
Characteristics of Epstein-Barr virus (EBV) seropositive and seronegative corona virus disease 2019 (COVID-19) patients

	Total (n = 67)	VCA IgM antibody		
		Positive (n = 37)	Negative (n = 30)	P value
Age, y	37 (30–52)	36 (28–52)	37 (31–52)	0.64
Female	35 (52.2)	17 (46.0)	18 (60.0)	0.25
Onset of symptom to hospital admission	4 (3–7)	4 (3–7)	4 (2–7)	0.94
Combined diseases	11 (16.4)	8 (21.6)	3 (10.0)	0.34
Symptoms				
Fever	41 (61.2)	27 (73.0)	14 (46.7)	0.03
Dry cough	35 (52.2)	23 (62.2)	12 (40.0)	0.07
Fatigue	31 (46.3)	14 (37.8)	17 (56.7)	0.12
Anorexia	16 (23.9)	11 (29.7)	5 (16.7)	0.21
Myalgia	18 (26.9)	11 (29.7)	7 (23.3)	0.56
Sore throat	11 (16.4)	5 (13.5)	6 (20.0)	0.70
Expectoration	11 (16.4)	7 (18.9)	4 (13.3)	0.78
Chest congestion	10 (14.9)	7 (18.9)	3 (10.0)	0.50
Vital signs				
Heart rate, bpm	78 (71–82)	78 (68–80)	78 (74–86)	0.51
Respiratory, bpm	19 (18–20)	18 (18–20)	19 (18–20)	0.42
Mean arterial pressure, mmHg	88 (86–92)	88 (86–92)	89 (86–94)	0.46
Temperature, °C	36.6 (36.5–37.0)	36.6 (36.5–37.0)	36.6 (36.5–37.0)	0.54

The most common of the initial symptoms were fever (41 [61.2%]), dry cough (35 [52.2%]), fatigue (31 [46.3%]), myalgia (18 [26.9%]) and anorexia (16 [23.9%]). Other symptoms such as sore throat, expectoration and chest congestion were less common (Table 1). CT abnormality was found in 63 (94.0%) COVID-19 patients, and ground-glass opacity (45 [72.6%]) was the commonest manifestation.

Among these COVID-19 patients, 37 (55.2%) patients were seropositive for anti-VCA IgM, 63 (94.0%) were seropositive for anti-VCA IgG and 64 (95.5%) were seropositive for anti-EBNA IgG. There were 36 (53.7%) patients were seropositive for anti-VCA IgM + anti-VCA IgG + anti-EBNA IgG + anti-EA IgM- or anti-VCA IgM + anti-VCA IgG + anti-EBNA IgG + anti-EA IgM or anti-VCA IgM + anti-EBNA IgG - anti-EBNA IgG + anti-EA IgM-, which indicated the recovery/reactivation of the EBV infection [16].

About other co-infection pathogens investigated in our study, only 8.1% (5/62) COVID-19 patients had positive anti-MP IgM and 1.6% (1/62) COVID-19 patients were positive for anti-RSV IgM. Among the patients with positive anti-MP IgM, 2 had PCR Capillary Electrophoresis Fragment Analysis for the MP, with negative result. 13 respiratory viruses were all negative in the tested 54 COVID-19 patients. The median CMV IgM antibody was 0.07 (IQR, 0.04–0.18; normal range, 0–12) AU/mL and CMV IgG antibody was 874.11 (IQR, 341.11-1518.74; normal range, 0–14) AU/mL in 59 COVID-19 patients.

Ebv Seropositive Vs Seronegative Covid Patients

When clinical symptoms in COVID-19 patients were compared with EBV VCA IgM antibody, EBV seropositive patients had a higher risk to report fever symptom than EBV seronegative patients (OR, 3.09; 95%CI, 1.11–8.56; P = 0.03). There were no significant association was detected between any other clinical symptoms, such as dry cough, fatigue, anorexia, myalgia and sore throat and EBV VCA IgM antibody in COVID-19 patients (Table 1). The key laboratory parameters in EBV seropositive and seronegative COVID-19 patients were shown in Table 2. The aspartate aminotransferase (AST) in EBV seropositive patients was significantly higher than that in EBV seronegative patients (P = 0.04). No other significant differences were detected in EBV seronegative and seropositive COVID-19 patients in blood routine examination and blood biochemistry results. CRP in EBV seropositive COVID-19 patients were higher than EBV seronegative patients (P = 0.02). There were no statistically significant differences in humoral immunity parameters between EBV seropositive and EBV seronegative COVID-19 patients. The values of humoral immunity parameters in all COVID-19 patients were all in the normal range. Although the CD8 count was lower in EBV seropositive COVID-19 patients than that in EBV seronegative patients, while the difference was not significant (P = 0.07). No statistically significant differences existed between cellular immunity parameters and the EBV VCA IgM antibody. The median counts of CD3, CD4, CD8, CD19 and CD16 + 56 were in the normal range.

Table 2

Laboratory findings of Epstein-Barr virus (EBV) seropositive and seronegative corona virus disease 2019 (COVID-19) patients

	Normal range	Total (n = 62)	VCA IgM antibody		
			Positive (n = 35)	Negative (n = 27)	P value
Blood Routine					
White blood cell count, $\times10^9$/L	3.5–9.5	4.27 (3.50–5.44)	4.10 (3.33–4.99)	4.53 (3.70–5.61)	0.11
Neutrophil count, $\times10^9$/L	1.8–6.3	2.38 (1.85–3.23)	2.21 (1.65–2.89)	2.59 (2.16–3.42)	0.11
Lymphocyte count, $\times10^9$/L	1.1–3.2	1.21 (0.98–1.64)	1.19 (0.98–1.73)	1.40 (0.99–1.52)	0.78
Monocyte count, $\times10^9$/L	0.1–0.6	0.43 (0.31–0.52)	0.41 (0.35–0.47)	0.45 (0.30–0.57)	0.42
Red blood cell, $\times10^{12}$/L	3.8–5.8	4.46 (4.16–4.85)	4.44 (4.13–4.82)	4.47 (4.18–4.85)	0.69
Platelet count, $\times10^9$/L	125–350	177 (147–227)	161 (145–203)	195 (165–237)	0.06
Blood biochemistry					
Alanine aminotransferase, U/L	7–50	18 (12–31)	24 (14–39)	17.5 (11–25)	0.12
Aspartate aminotransferase, U/L	13–40	22 (18–28)	24 (19–30)	20.5 (17–24)	0.04
Total bilirubin, umol/L	0–23	8.3 (6.1–12.0)	8.6 (6.6–10.7)	8.3 (6.1–12.8)	0.73
Creatinine, μmol/L	41–97	59 (49–72)	63 (51–72)	53.5 (48–69)	0.42
Blood urea nitrogen, mmol/L	2.6-8.0	4.15 (3.52–4.71)	4.19 (3.73–4.72)	3.95 (3.37–4.63)	0.52
Potassium, mmol/L	3.5–5.3	4.08 (3.88–4.37)	4.08 (3.84–4.30)	4.10 (3.91–4.39)	0.61
Creatine kinase, U/L	40–310	73.5 (49.0-99.5)	75 (47–101)	67 (51–90)	0.33
Lactate dehydrogenase, U/L	120–250	193 (171–234)	204 (175–265)	185.5 (169.5-217.5)	0.33
Glucose, mmol/L	3.9–6.1	4.86 (4.52–5.54)	4.86 (4.61–5.54)	4.83 (4.49–5.46)	0.50

	Normal range	Total (n = 62)	VCA IgM antibody		
			Positive (n = 35)	Negative (n = 27)	P value
Infection-related biomarkers					
C-reactive protein, mg/L	0–10	4.85 (0.5–17.4)	8.2 (0.5–24.7)	2.0 (0.5–5.7)	0.02
Humoral immunity					
Serum IgG, g/L	8–16	11.10 (9.89–13.10)	10.90 (9.89–13.1)	11.20 (10.20–12.60)	0.96
Serum IgM, g/L	0.4–3.45	0.959 (0.688–1.290)	1.030 (0.688–1.440)	0.924 (0.722–1.150)	0.46
Serum IgA, g/L	0.76–3.9	1.93 (1.54–2.72)	1.94 (1.54–2.72)	1.92 (1.60–2.64)	0.86
C3, g/L	0.81–1.6	0.879 (0.740–0.983)	0.878 (0.738–0.988)	0.890 (0.774–0.963)	0.69
C4, g/L	0.1–0.4	0.260 (0.213–0.320)	0.266 (0.213–0.321)	0.243 (0.216–0.319)	0.52
Cellular immunity					
CD3 count, /uL	723–2737	752 (589–1047)	746 (569–1006)	871 (669–1047)	0.34
CD4 count, /uL	404–1612	429.5 (308–565)	406 (308–628)	469 (366–545)	0.57
CD8 count, /uL	220–1129	276.5 (194–424)	254 (188–350)	310 (235–480)	0.07
CD19 count, /uL	80–616	137.5 (103–179)	139 (98–186)	136 (114–168)	0.97
CD16 + 56 count, /uL	84–724	155 (97–274)	154 (86–275)	156 (115–248)	0.99
CD4/CD8, ratio	0.9-2.0	1.56 (1.14–2.12)	1.64 (1.24–2.18)	1.51 (1.07–1.66)	0.10

60 (89.6%) patients received interferon alpha inhalation, 59 (88.1%) patients were given empirical antibiotic treatment, 37 (55.2%) were given antiviral treatment, 32 (47.8%) patients received systematic corticosteroid treatment and 28 (41.8%) patients were given gamma globulin therapy (Table 3). EB seropositive patients had a higher portion of corticosteroid use than the EB seronegative patients (P = 0.03). Of the 67 COVID-19 patients, 65 (97.0%) were discharged before March 16, 2020 according to the discharged criteria. The median recovery time for COVID-19 patients was 34 days (IQR, 23–42 days), with

36 days for EBV seropositive patients (IQR, 25–42 days) and 34 days for EBV seronegative patients (IQR, 15–23 days). About the recovery time, the difference was not significant between the EBV seropositive patients and EBV seronegative patients (P = 0.36).

Table 3
Treatments of Epstein-Barr virus (EBV) seropositive and seronegative corona virus disease 2019 (COVID-19) patients

	Total (n = 67), n (%)	VCA IgM antibody		
		Positive (n = 37), n (%)	Negative (n = 30), n (%)	P value
Antiviral	37 (55.2)	22 (59.5)	15 (50.0)	0.44
Antibiotics	59 (88.1)	32 (86.5)	27 (90.0)	0.95
Corticosteroid	32 (47.8)	22 (59.5)	10 (33.3)	0.03
Gamma globulin	28 (41.8)	19 (51.4)	9 (30.0)	0.08
Interferon alpha inhalation	60 (89.6)	34 (91.9)	26 (86.7)	0.77
Oxygen inhalation	8 (11.9)	7 (18.9)	1 (3.3)	0.11

Discussion

In this study, we attempted to jointly explain the differences in the clinical characteristics of COVID-19 patients from the perspective of other pathogens coinfections. This study, to our best knowledge, is the first study reporting the EBV coinfection in COVID-19 patients and also the first evaluation of clinical immune function to detect the possible mechanism for understanding different clinical characteristics in COVID-19 patients. The main findings in our study were as follows:1) more than half of COVID-19 patients were positive for EBV VCA IgM antibody; 2) EBV VCA IgM antibody was associated with fever, higher CRP and higher AST; 3) the EBV seropositive COVID-19 patients were more likely to be given corticosteroid therapy by doctors; 4) The CD8 in EBV seropositive COVID-19 patients was a litter less than that in EBV seronegative patients.

EBV is a ubiquitous human virus with a productive lytic cycle and a latent phase. The acute infection is mainly asymptomatic in children and the latent infection can be last for the whole life [17]. Specific antibodies are induced after EBV infection, including VCA IgM, IgG, EBNA IgG and EA IgM, IgG. The products of lytic infection include the EA complex and VCA. Primary infections occur mostly in children, and in general, serum positive for anti-VCA IgM indicates acute infection [17]. The VCA IgG antibody appears at the acute infection stage, and remain positive for life [17]. EBNA IgG antibody is indication of past infection [17]. Latent EBV can be reactivated and become a lytic infection, expressing anti-VCA IgM

[18]. EBV reactivation has been reported in psychological stress of various type because of impaired the cellular immune function, including student examination stress [19], attachment anxiety [20] and loneliness [21]. EBV reactivation also found in autoimmune diseases [8]. In our study, 55.2% COVID-19 patients had positive VCA IgM antibody, indicating an EBV reactivation happened in COVID-19 patients. The VCA IgM antibody generally disappeared 1–2 weeks after onset [17], and we could not confirm the times of EBV infection and SRAS-CoV-2 infection. To reduce the possibility of false negative VCA IgM antibody, we only included COVID-19 patients with onset time within 2 weeks. The specificity of positive VCA IgM antibody also need to be verified. It may have cross-reactivities with CMV and other respiratory pathogens. In our study, negative CMV IgM antibody were found in COVID-19 patients. Other respiratory pathogens were also tested, only 8.1% COVID-19 patients had positive anti-MP IgM and 1.6% were positive for anti-RSV IgM. While 2 had PCR Capillary Electrophoresis Fragment Analysis for the MP with negative result. Thus the possibility of false positive is small. During lytic stage of EBV infection, CD8 T cells dominant the response for EBV infection [22]. Liu et al. found a decrease in CD8 count in the laboratory examination of 12 COVID – 19 patients [23]. In EBV seropositive COVID-19 patients, we found that CD4/CD8 increased while CD8% and CD8 counts decreased. Increased CD4/CD8 count is more common in autoimmune diseases, viral infections and allergic reactions. Our data suggested a highly activated immune response to EBV reactivation.

Similar to previous study, the typical symptoms on admission of our COVID-19 patients were fever, dry cough, fatigue and myalgia [12, 24], indicating the representativeness of our COVID-19 patients. When clinical symptoms in COVID-19 patients were compared with EBV seropositive antibody, we found that EBV seropositive COVID-19 patients had a 3.09-fold risk of having a fever symptom than EBV seronegative. This result also indicated a coinfection of EBV with SRAS-CoV-2 in COVID-19 patients.

CRP, as an acute reactant, is produced in bacterial infection or inflammation [25]. Some studies reported that CRP was higher in the severe group than in the non-severe group [26, 27], and may also be a potential predictor of disease severity [28]. Other studies reported that cytokine storms might occur in COVID-19 patients, and the pro-inflammatory cytokine Th1, Th2 and Th17 were elevated [29]. In our study, the CRP in the EBV seropositive COVID-19 patients was higher than that in the seronegative patients, indicating a powerful inflammatory response in EBV seropositive COVID-19 patients. Meanwhile, EBV seropositive patients had higher AST levels than seronegative patients in our study. Zhao et al. [30] reported that had higher levels of AST was found in COVID-19 patients when compared with pneumonia patients not infected with SARS- CoV-2. Higher levels of AST and CRP were also found in refractory patients compared with general COVID-19 patients [31]. Higher use of corticosteroid, prescribed when patients suffered from CT scan exacerbation or persistent fever exceeding 39℃, was found in EBV seropositive patients. All of this indicated that EBV reactivation may associated with the severity of COVID-19.

Therefore, we hypothesized that EBV seropositive COVID-19 patients may need more time to recovery than the seronegative patients. We analyzed the recovery time between EBV seropositive COVID-19 patients and seronegative patients. The recovery time is a little more in EBV seropositive COVID-19

patients, while the difference was not significant. The reason of this negative result may be that most of our included COVID-19 patients were mild cases.

Our study had several limitations. First, our study was a retrospective design, we could not confirm the time of EBV infection. Second, the sample size in our study was relatively small. Third, most COVID-19 patients did not test the EBV DNA, so we could not assess the viral loads in our study. Forth, most included patients were mild cases, we could not analyze the associations between anti-EBV antibodies and the severity of COVID-19.

Conclusions

In summary, our study showed that EBV acute infection was found in COVID-19 patients. EBV seropositivity was associated with fever and increased inflammation in COVID-19 patients. EBV reactivation may affected the treatment of COVID-19. The underlying mechanism of how EBV reactivates and affects the COVID-19 needs to be investigated.

Declarations

Ethics approval and consent to participate

The study was approved by the ethical committee board of Renmin Hospital of Wuhan University (WDRY2020-K073).

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

TC and JYS conceptualized the paper. TC, HLL and HMZ collected the data. TC, JYS and HLL performed the literature search. TC, JYS, HLL, CZC and HMZ analyzed and interpreted the data. TC and JYS wrote

the drafting of this manuscript with all authors providing critical feedback and edits to subsequent revisions. All named authors have read and approved the manuscript, contributed significantly to the work, and accepted responsibility for the manuscript's contents.

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Figures

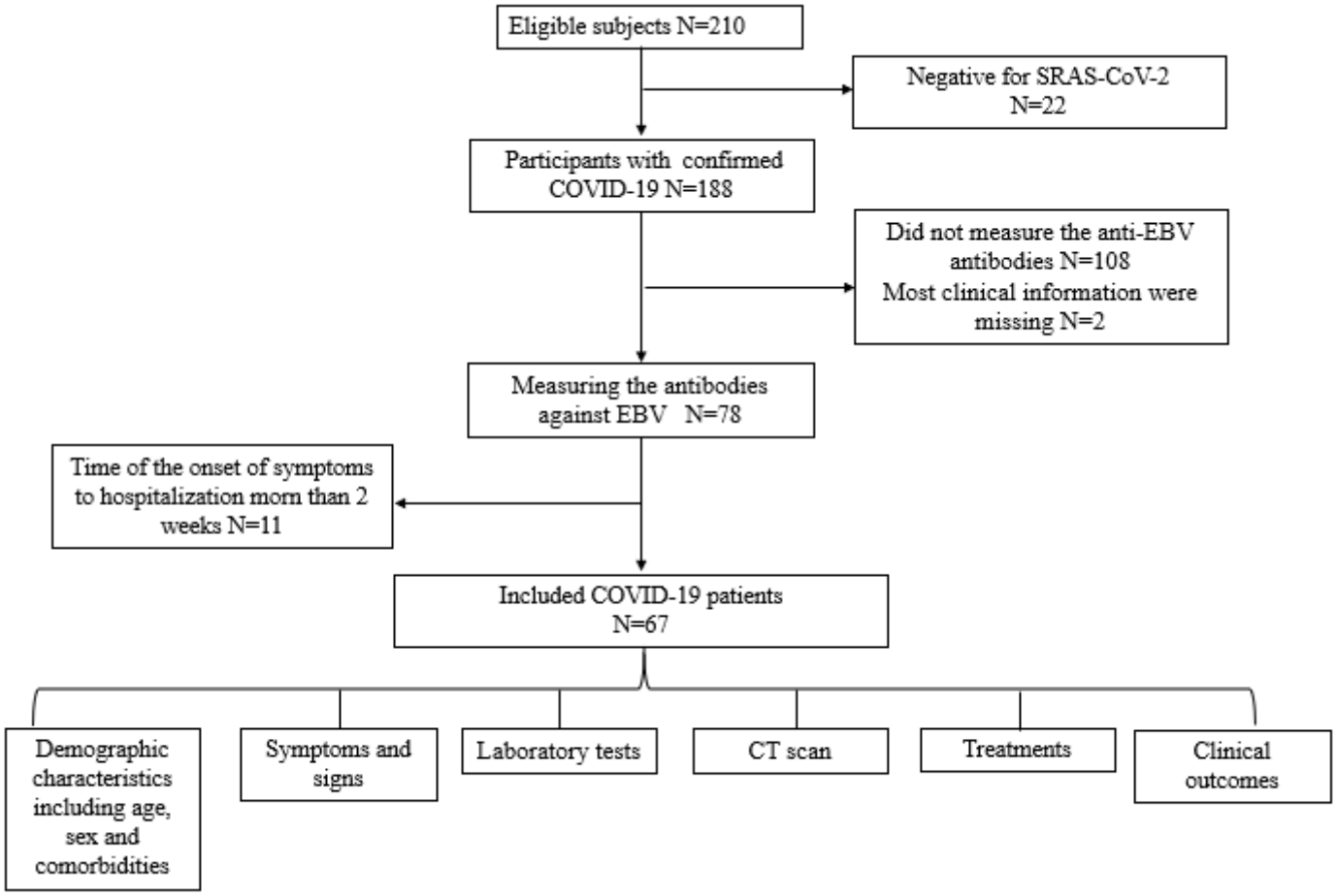

Figure 1

The workflow of corona virus disease 2019 (COVID-19) patients' inclusion and exclusion criteria