Severity of thrombocytopenia in COVID-19 patients with Epstein-Barr virus and cytomegalovirus coinfections

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Abstract

***Introduction*: This study aimed to investigate the prevalence of viral coinfections in hospitalized patients and their potential association with platelet count (PLT) during hospitalization. Additionally, the study explored the gender and ethnic distribution of the participants, providing valuable insights into the diversity of the patient population.**

***Methods*: Of 994 patients with coronavirus disease 2019 (COVID-19), a total of 354 patients were included in the study, with 54.8% males and 45.2% females. The ethnic distribution consisted of 52.8% Hispanic, 21.8% Caucasian, 19.5% African American, and 4.5% classified as other. Viral coinfections were analyzed during hospitalization, and groups were analyzed based on the degree of thrombocytopenia, which was classified into mild (100-150 x 10^3/µl), moderate (50-99 x 10^3/µl), and severe (<50 x 10^3/µl).**

***Results*: Among the participants, 46.3% had viral coinfections during hospitalization. Active Epstein-Barr virus (EBV) coinfection was the most prevalent (18.6%), followed by active Cytomegalovirus (CMV) coinfection (2.3%), and dual EBV/CMV coinfection (3.1%). The median platelet level on admission was 149,500 (121,000-224,250) and the median lowest platelet count during hospitalization was 131,500 (85,000-188,250).**

The median Acute Physiology and Chronic Health Evaluation (APACHE) II score for the total population was 11 upon admission. Notably, the median APACHE II score for severity of illness upon admission for the total population was 11, ranging from 7-18, while mild was 9 (6-14), moderate was 10 (6.5-14), and severe being associated with severity scores. While the results suggest certain trends, future research with larger and more diverse populations is needed to draw definitive conclusions.

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Introduction

In December 2019, the first case of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) known as coronavirus disease 2019 (COVID-19) emerged in Wuhan, China. The virus spread so rapidly worldwide that the World Health Organization (WHO) declared a pandemic in March 2020. (1) This virus has three modes of transmission: airborne, droplet, and contact, and it has an incubation period between 2 to 14 days. The incubation period will vary on the different types of variants such as the Delta, and Omicron. COVID-19 has a wide variety of symptoms, but the most commonly reported were fever/chills, new loss of taste and smell, cough, shortness of breath, sore throat, and fatigue. (2,3) Once patients are admitted to a hospital with

COVID-19 pneumonia, a trend of different abnormal laboratory results is noticed. For example, thrombocytopenia, reactivation of Epstein-Barr virus (EBV) and Cytomegalovirus (CMV) infection, and low levels of CD4/CD8. (4,5)

Thrombosis that occurs in COVID-19 is a serious complication, a critical aspect of pathogenesis in the disease progression, and a bad prognostic indicator in critical care. Although thrombocytopenia is uncommon upon initial presentation, it may also reflect disease severity due to the ability of SARS-CoV-2 to activate platelets. This occurs directly through the spike protein-angiotensin converting enzyme 2 (ACE2) interaction and indirectly by coagulation and inflammation activation. (6,7) Dysregulation in both innate and adaptive immune systems is another critical factor that causes thrombosis and thrombocytopenia in COVID-19. (8)

This study aimed to investigate the relationship between thrombocytopenia levels in two distinct groups of hospitalized COVID-19 patients and its association with viral coinfections. The primary focus of this study was to explore the potential influence of thrombocytopenia on the clinical outcomes of hospitalized patients with COVID-19.

Materials and methods

Study design and participants

This retrospective observational study was conducted using medical records of patients diagnosed with COVID-19 infection from March 2020 to February 2022**.** The study population consisted of patients admitted to a single center in Houston, Texas, USA.

This study was conducted following ethical guidelines and was approved by the Institutional Review Board (IRB) before data collection. Patient information was anonymized and handled with strict confidentiality to protect individual privacy.

Inclusion criteria for both groups are as follows: (a) Laboratory-confirmed COVID-19 infection via reverse transcription polymerase chain reaction (RT-PCR) or antigen test; (b) Age above 18 years; and (c) Availability of complete medical records, including platelet counts and relevant laboratory results.

Group 1 consisted of patients who were positive for COVID-19 infection, with concurrent thrombocytopenia and coinfection with CMV or EBV. In contrast, Group 2 comprised COVID-19-positive patients with thrombocytopenia but without any additional coinfections. These groups were analyzed based on the degree of thrombocytopenia, which was classified into mild (100-150 x 10^3/μl), moderate (50-99 x 10^3/μl), and severe (<50 x 10^3/μl).

Data collection

Data was collected from electronic medical records and laboratory databases of the hospital**.** Demographic information, clinical characteristics, and laboratory results were retrieved for each patient. Specific data collected included age, gender, comorbidities, length of hospital stay, admission to the intensive care unit (ICU), mechanical ventilation requirements, platelet count, EBV and CMV test results, and CD4/CD8 levels.

Platelet count and thrombocytopenia assessment

The primary outcome of interest was the level of thrombocytopenia in each patient group. Thrombocytopenia was defined as a platelet count below 150,000 per microliter (μl), in accordance with standard clinical definitions. (9) Platelet counts were collected from the first laboratory test conducted after admission for COVID-19.

EBV and CMV detection

EBV and CMV infection status were determined using specific serological assays, such as enzyme-linked immunosorbent assay (ELISA), which detected antibodies against viral antigens. The presence or absence of EBV and CMV coinfection was recorded for each patient in Group 1.

Data analysis

Continuous data are expressed as median values with interquartile ranges (p25th-p75th) and were analyzed using the Mann-Whitney U test. Categorical variables are expressed as numerical values and percentages; these were evaluated by the Chi-square test and Fisher's exact test. Bivariate analysis, multiple analysis, and multiple logistic regression were used to determine mortality risk factors in patients with COVID-19 and viral reactivation. Analysis was performed with IBM SPSS version 23 software, and statistical significance was determined by $p \leq 0.05$.

Statistical analysis, which was performed using descriptive statistics, was used to summarize the demographic and clinical characteristics of both patient groups.

Moreover, regression analysis was conducted to explore potential associations between thrombocytopenia severity and other variables, including age, gender, comorbidities, length of hospital stay, admission to ICU, mechanical ventilation, and EBV/CMV coinfection.

Results

Of 994 critically ill patients admitted with COVID-19 to our institution, a total of 354 patients were in-

cluded in this study. Among the participants, 194 (54.8%) were male, 160 (45.2%) were female, 77 (21.8%) were Caucasian, 187 (52.8%) were Hispanic, 69 (19.5%) were African American, and 16 (4.5%) were classified as other. When analyzing the presence of viral coinfection during hospitalization, 164 patients (46.3%) had a coinfection (Group 1), in comparison to Group 2 which 190 patients (53.7%) had no coinfection at all (**Table 1**). Out of those 164 patients, 66 (18.6%) had an active EBV coinfection, 8 (2.3%) had an active CMV coinfection, and 11 (3.1%) had dual EBV/CMV coinfection (**Table 2**). When analyzing the presence of inflammatory markers in patients with thrombocytopenia and EBV, CMV, and EBV/CMV coinfection, there was no correlation between inflammatory markers levels and thrombocytopenia (**Table 3**). Regarding the severity scores, out of the 354 patients, the median APACHE II score for severity of illness upon admission for the total population was 11 (ranging from 7-18), while in mild was 9 (6-14), in moderate was 10 (6.5-14), and in severe was 10.5 (7-13.75) (**Table 4**). The median platelet level on admission was 149,500 (121,000-224,250) and the median lowest platelet count during hospitalization was 131,500 (85,000-188,250) (**Table 5**).

Discussion

The data presented in this study provides valuable insights into the relationship between platelet counts, viral co-infections, and the clinical outcomes of hospitalized COVID-19 patients. Group 1, consisting of patients with reactivation of EBV and CMV, exhibited a higher incidence of thrombocytopenia across all severity levels compared to Group 2, which comprised patients without reactivation of EBV and CMV. This intriguing finding suggests that coinfection with EBV and CMV might contribute to the development of thrombocytopenia in COVID-19 patients, potentially influencing disease progression and patient outcomes.

The higher mortality rate observed in Group 1 compared to Group 2 further underscores the significance of thrombocytopenia as a crucial element influencing the severity and outlook of COVID-19. Thrombocytopenia, which is characterized by unusually low levels of platelets, can result in compromised blood clotting and an elevated susceptibility to bleeding. (10-12) Within the context of COVID-19, the reasons behind thrombocytopenia are complex. Firstly, SARS-CoV-2 has the ability to directly trigger platelets via its spike protein's interaction with angiotensin-converting enzyme 2 (ACE2). This interaction caused by the virus stimulates platelet activation and can contribute to the formation of small blood clots or microthrombi. (13,14) Additionally, the indirect activation of platelets through coagulation and inflammation pathways adds another layer of complexity to the disease. COVID-19 is associated with a pro-inflammatory state and dysregulated coagulation, leading to a hypercoagulable state known as thrombosis. (15) The interplay among active platelets, clotting factors, and the immune response establishes a conducive setting for blood clot creation in different organs, such as the lungs, heart, and brain. This situation has the potential to worsen the disease's seriousness. (16)

Furthermore, the dysregulation of both the innate and adaptive immune systems in COVID-19 patients plays a significant role in the pathogenesis of thrombosis and thrombocytopenia. (17) The virus can trigger an overactive immune response, leading to a cytokine storm, where a surge of inflammatory molecules can further contribute to endothelial dysfunction and blood clot formation. (18)

This study's findings raise critical questions for future research, such as the mechanisms underlying the reactivation of EBV and CMV in COVID-19 patients and their potential contribution to the thrombotic complications observed in Group 1. Understanding the interplay between these viruses and their impact on platelet function and immune response may pave the way for targeted therapeutic interventions to reduce the severity of thrombocytopenia and improve patient outcomes. (19, 20)

The identification of thrombocytopenia as a potential predictor of adverse clinical outcomes in COVID-19 patients calls for vigilant monitoring of platelet counts in hospitalized individuals. (21) Early recognition of thrombocytopenia can aid in risk stratification and prompt the implementation of appropriate interventions to prevent further complications. (22)

The management of thrombocytopenia in COVID-19 patients should involve a multidisciplinary approach, including hematologists, infectious disease specialists, and intensivists. Tailored treatment strategies, such as antiviral therapies, anti-inflammatory agents, and anticoagulation measures, need to be explored to address the complexities of thrombocytopenia and its underlying mechanisms. (23)

Conclusions

This study sheds light on the intricate relationship between thrombocytopenia levels and clinical outcomes in hospitalized COVID-19 patients who have viral coinfections, particularly in those with reacti-

vation of EBV and CMV. The findings emphasize the critical role of platelets in the pathogenesis of COVID-19 and the importance of understanding the underlying mechanisms of thrombocytopenia. By further exploring the interactions between viral coinfections, platelet activation, and immune dysregulation, we can work towards more targeted and effective interventions to improve patient care and ultimately combat the devastating impact of COVID-19. Continued research and collaboration among the scientific community are essential in this ongoing battle against the pandemic.

Conflicts of interest

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Table 1. Groups of patients with thrombocytopenia severity with and without reactivation of EBV/CMV infection

Level of thrombocytopenia	Group1 Reactivation of EBV and CMV	Group 2 No reactivation of EBV and CMV
Normal platelet count	98	132
Mild (100-150 x 10^3/μl)	44	59
Moderate (50-99 x 10^3/μl)	47	26
Severe (<50 x 10^3/μl)	29	10
Total number of patients	218	227

Legend: EBV=Epstein-Barr Virus; CMV=cytomegalovirus. This Table provides insight into the relationship between thrombocytopenia severity and reactivation of EBV and CMV infection in hospitalized COVID-19 patients.

Table 2. Patients with EBV, CMV, EBV/CMV coinfection and mortality rate

Thrombocytopenia severity	Mild	Moderate	Severe	p-value
EBV infection/reactivation	32 (21.9%)	2 (8%)	1 (16.5%)	<0.001
CMV infection/reactivation	5 (3.4%)	0	0	=0.113
EBV and CMV infection or reactivation	4 (2.7%)	1 (4%)	0	<0.001
Death	42 (28.8%)	7 (28%)	1 (16.7%)	<0.001

Legend: EBV=Epstein-Barr Virus; CMV=cytomegalovirus. This Table provides an overview of hospitalized COVID-19 patients with thrombocytopenia categorized by their coinfection status and their associated mortality rates. It aims to show the impact of these viral infections on patient outcomes.

Table 3. Inflammatory markers in patients with thrombocytopenia and EBV, CMV, EBV/CMV coinfection

Thrombocytopenia severity	Mild	Moderate	Severe	p-value
Ferritin level on admission (ng/ml)	387 (165-773)	387 (146.7-439.1)	492.3 (269.975-1600)	=0.008
CRP level on admission (mg/l)	48.7 (27.05-109.5)	62.9 (23.5-106.25)	57.8 (21.7-93.9)	=0.008
CPK level on admission (µg/l)	151.5 (52.75-296.25)	180 (98-532)	58 (42.75-84.75)	=0.437
ESR level on admission (mm/hr)	39.5 (22-65.25)	42.5 (23.75-69.5)	44.5 (7-96.75)	=0.003
$CD4^+$ first recorded value (cell/mm^3)	291	112 (78-374)	334.6 (176-529)	<0.001
$CD8^+$ first recorded value (cell/mm^3)	131 (92-265)	80 (35-410)	194 (125.5-278)	<0.001
IL-6 first recorded value (cell/mm^3)	12.4 (5.35-34.92)	11.15 (3.15-46)	4.3 (2.45-89.9)	<0.001
IL-10 first recorded value (cell/mm^3)	17.6 (11.5-29.1)	18.3 (7.8-33)	12.5 (6.7-45.9)	<0.001

Legend: EBV=Epstein-Barr Virus; CMV=cytomegalovirus; CRP=C-reactive protein; CPK=creatine phosphokinase; ESR=erythrocyte sedimentation rate; IL=interleukin. This Table showcases the levels of inflammatory markers in hospitalized COVID-19 patients and EBV/CMV infections.

Table 4. Severity scores in patients with thrombocytopenia and EBV, CMV, EBV/CMV coinfection

Thrombocytopenia severity	Mild	Moderate	Severe	p-value
APACHE score	9 (6-14)	10 (6.5-14)	10.5 (7-13.75)	<0.001
Highest APACHE score	12 (7-15)	13 (9-25)	10.5 (7-19)	<0.001
SOFA score	3 (2-4)	4 (3-6)	4.5 (1.75-6.5)	<0.001
Highest SOFA score	2 (1-3)	6 (4-10.5)	6 (1.75-10)	<0.001

Legend: EBV=Epstein-Barr Virus; CMV=cytomegalovirus; APACHE=Acute Physiology and Chronic Health Evaluation; SOFA=Sequential Organ Failure Assessment. This Table presents severity scores associated with thrombocytopenia in hospitalized COVID-19 patients with EBV/CMV coinfection. The severity scores provide insight into the impact of coinfection and the overall severity of thrombocytopenia in these patients.

Table 5. Laboratory biomarkers for coagulation in patients with EBV, CMV, EBV/CMV coinfection

Thrombocytopenia severity	Mild	Moderate	Severe	p-value
Albumin level on admission (g/dl)	3.7 (3.4-4)	3.4 (2.9-3.7)	3.3 (2.72-3.65)	=0.043
Platelet level on admission (x 10^3/ul)	126 (109-139)	56 (46.5-66.5)	13.8 (8.25-16.5)	=0.008
Prothrombin time on admission (sec)	10.9 (10.6-11.7)	12.9 (10.9-16.97)	12.05 (10.77-13.02)	=0.017
Fibrinogen on admission (mg/dl)	429 (340-504.5)	374.5 (236-500.75)	464 (364.5-624.5)	<0.001
D-dimer level on admission (mg/l)	0.400 (0.2375-0.74)	0.97 (0.42-5.8)	2.47 (0.545-4.5)	<0.001
Activated partial thromboplastin time on admission (sec)	31 (28-34)	33 (28-35.5)	30.5 (25.25-36.75)	<0.001

Legend: EBV=Epstein-Barr Virus; CMV=cytomegalovirus. This Table presents biomarkers for coagulation in hospitalized COVID-19 patients with thrombocytopenia and EBV/CMV coinfection. This aims to show potential links with viral infection, low platelet, and coagulation changes.

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