Am J Blood Res 2022;12(2):54-59
www.AJBlood.us /ISSN:2160-1992/AJBR0141658

Original Article

Dipyridamole does not have any additive effect on the prevention of COVID-19 coagulopathy

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Received January 10, 2022; Accepted February 23, 2022; Epub April 15, 2022; Published April 30, 2022

Abstract: Objective: Severe acute respiratory syndrome (SARS) coronavirus 2 (SaRS-Cov-2) associated respiratory disease (COVID-19), announced as a pandemic, is a multisystem syndrome. SARS-CoV-2 directly infects and damages vascular endothelial cells, which leads to microvascular dysfunction and promotes a procoagulant state. Dipyridamole (DP) acts as a reversible phosphodiesterase inhibitor and is used mainly as an antiplatelet agent. It is hypothetised that it has possible activities in COVID-19. Design and Methodology: We report our retrospective, real-world results of DP added to low-molecular weight heparin (LMWH) in the treatment of 462 clinically diagnosed and hospitalized COVID-19 patients. We compared anticoagulation with and without DP addition with no administration of anticoagulation in the same time frame. The primary outcome was proven or highly suspected coagulopathy within 30 days of hospitalization. Results: Definitive coagulopathy has been diagnosed in 3 (3.5%) of 85 LMWH administered patients and 7 (2.13%) of 328 DP + LMWH received patients (P=0.456). Five cases with definitive coagulopathy were not initiated any anticoagulation at the time of the event. The multivariate analysis showed that DP addition to the anticoagulant approach did not have any impact on the risk of demonstrated coagulopathy and highly-suspected coagulopathy. Conclusion: We think that our clinical experience is valuable in showing the real-life results of DP + LMWH treatment in COVID-19. This approach did not affect the coagulopathy rate. Our data did also not document an additive effect of DP in the COVID-19 outcome. Prospective controlled trials would give more convincing results regarding the role of DP in COVID-19 endothelial dysfunction and clinical outcome.

Keywords: SARS-CoV-2, COVID-19 pneumonia, coagulopathy, dipyridamole, low-molecular-weight heparin

Introduction

After recognition and declaration of the severe acute respiratory syndrome (SARS) coronavirus 2 (SaRS-Cov-2) associated respiratory disease (COVID-19) as a pandemic, detailed observations and researches were published afterward and time progress contributed to the discovery of COVID-19 pathophysiology, resulting in the better management of the disease [1]. The clinical course showed variability in severity and increased mortality primarily in the elderly and/or in patients with comorbidities was reported [2-4].

It is now established that severe COVID-19 is a multisystem syndrome. SARS-CoV-2 directly infects and damages vascular endothelial cells, which leads to microvascular dysfunction and promotes ischemia, inflammation, and a procoagulant state [5, 6]. Increased pro-inflammatory cytokines (IL-1, IL-6, and TNF) and ferritin levels are associated with platelet activation, which contributes to amplification of so-named COVID-19 associated coagulopathy [7, 8]. Both antithrombotic and anti-inflammatory treatment would be the ideal therapeutic approach in this scene. However, anticoagulant treatment with low molecular weight heparin (LM-

WH) was proven to be associated with decreased 28-day mortality in patients with a sepsis-induced coagulopathy score (SIC) equal or more than 4 or D-dimer increase to six times of the upper limit of normal [9, 10].

Dipyridamole (DP) is an old drug, acting as a reversible phosphodiesterase (PDE) inhibitor and used mainly as an antiplatelet agent. Prevention of NETosis by promoting 3',5'-cyclic adenosine monophosphate (cAMP) generation in neutrophils, and broad-spectrum antiviral activity (especially against positive-stranded RNA viruses) are the possible additive activities of DP in COVID-19 [11, 12]. DP may also potentiate vascular-protective effects of endothelium-derived nitric oxide [13]. Here, we report the retrospective analysis of our single-center real-world experience of DP in COVID-19 treatment.

Methodology

The institution established a scientific team just after the announcement of COVID-19 as a pandemic. Nose and throat swab was obtained from patients with suspected signs and symptoms of COVID-19 and RT-PCR analysis defined by the World Health Organization (WHO) was performed. Additionally, blood chemical analysis for inflammation and thoracic computed tomography (CT) were carried out [14]. CT findings were stratified as mild, moderate, and severe according to the five lobe scores [15, 16].

The treatment approach was designed according to the national COVID-19 treatment guideline. Patients with moderate or severe COVID-19 pneumonia were all hospitalized.

During follow-up, newly developed and documented arterial and/or venous thrombosis or disseminated intravascular coagulation (DIC) were recorded as COVID-19 coagulopathy. The clinical situations such as abrupt severe dyspnea and/or hypotension, accompanied by decreased oxygen saturation and striking D-Dimer elevation, however lacking documented thrombosis imaging, were stratified as highly suspected COVID-19 coagulopathy.

All patients received favipiravir according to the national guideline. Anticoagulant prophylaxis with LMWH was recommended in hospitalized patients and/or in cases with a D-dimer level ≥1000 U/ml. Enoxaparin was preferred as LMWH agent (1 mg/kg, q2d). At the third week of our first COVID-19 case detection, a DP (bid 75 mg orally for two months) has been added to the treatment protocol as the institution's initiative. The steroid agents were used as first-line for the treatment of macrophage activation syndrome and, anti-cytokine molecules either as anti-interleukin-1 or anti-interleukin-6 were used as the second line treatment.

COVID-19 diagnosis was primarily based on radiologic evaluation [16]. Thereby, SARS-Cov-2 PCR positive and negative patients were both included in the study. Patients having comorbidities associated with coagulopathy and/or those with history of thrombosis which may affect the COVID-19 outcome were excluded from the analysis.

We compared anticoagulation with and without DP, with no administration of anticoagulation in the same time frame. The primary outcome was proven or highly suspected coagulopathy within 30 days of hospital admission.

Continuous variables were presented as median and, the number of the categorical variable was given. The differences between groups were analyzed with χ^2 test. The risk factors were evaluated in the univariate and multivariate logistic regression models. The propensity score matching method was administered to find the best matching cohort for patients who did not receive LMWH, and similarly for those who did not receive DP. Statistical analysis was performed in the STATA 13 software.

Results

We retrospectively analyzed our real-world COVID-19 cohort. A total of 510 patients were hospitalized with COVID-19 diagnosis between March 11th and May 5th of 2020. Forty-eight patients were excluded from the analysis due to insufficient data (n=16), active and mostly metastatic malignancy (n=11), pregnancy (n=6), suspicious COVID-19 diagnosis (n=5), high d-dimers levels secondary to pathologic conditions other than COVID-19 (n=3), thrombosis unrelated with COVID-19 (n=3), extremely high levels of ferritin irrelevant with Covid-19 (n=2) and, being in the post-operative period (n=2).

Table 1. Demographic characteristics, initial signs, and symptomatology

	n=462
Median Age (range)	56 (23-98)
Sex	
Female (%)	178 (38.5%)
Male (%)	284 (61.5%)
Initial symptoms	
Fatigue and myalgia	432 (94%)
Cough	389 (84%)
Fever	224 (72%)
Dyspnea	198 (43%)
Nausea	71 (15%)
Diarrhea	54 (12%)
Anosmia	38 (8%)
Sputum	14 (3%)
Initial vital signs	
Median saturation on pulse oximetry (range)	96% (70-100)
Median systolic blood pressure (range)	130 (80-250)
Median diastolic blood pressure (range)	75 (50-136)
Median pulse rate (range)	93 (66-190)
Median respiratory rate (range)	18 (12-36)
Comorbid conditions	
Hypertension	182 (40%)
Diabetes mellitus	100 (22%)
COPD or Asthma	56 (12%)
Coronary artery disease	51 (11%)
Congestive heart failure	30 (6.5%)
Solid malignancy	22 (5)
Hematologic malignancy	13 (3%)
The median number of comorbidities (range)	1 (0-6)
COVID RT-PCR positive vs negative	285 vs 177

Among these 48 excluded patients, three patients had proven coagulopathy, and ten patients had highly suspected coagulopathy.

Four-hundred and sixty-two patients were included in the final analysis. The median age was 56 years (range: 23-98 years). The gender distribution showed male preponderance. SARS-CoV2 positivity was proven by RT-PCR in 62% of the cohort (n=285) (**Table 1**). The demographic features, the laboratory values at the initial presentation, and at the time of the peak inflammatory period were given in **Table 1**. Thirteen patients did not receive anticoagulation. DP was used alone (n=36) or with LMWH (n=328). Eighty-five patients received LMWH only.

The overall definitive coagulopathy rate and higly-suspected coagulopathy were 3.25% (n=15) and 10.2% (n=47), respectively.

Seven of 15 definitive coagulopathy cases had arterial thrombosis, whereas 4 had venous thromboses and, 4 had DIC (**Table 2**). Arterial thromboses were observed as myocardial infarction (n=5), arterial thrombosis in the extremity (n=1), and cerebrovascular event (n=1). The median time from COVID-19 diagnosis to arterial thrombosis was 4 days (range: 1-16). Venous thrombosis developed at a median of 10 days (range: 13-33), presenting as catheter-related thrombosis (n=1), pulmonary embolism (n=1), cannula thrombosis of extracorporeal membrane oxygenation (n=1) and, deep vein thrombosis (n=1).

All 4 cases with DIC had proven bacterial septicemia (one case with Pseudomonas, one with Klebsiella and Enterococcus, one with Pseudomonas and Acinetobacter, one with pan-resistant Klebsiella, and acinetobacter).

Definitive coagulopathy has been diagnosed in three of 85 LMWH administered patients (3.5%) and 7 of 328 DP + LMWH received patients (2.13%) (P=0.456). Five cases with definitive coagulopathy were not initiated any anticoagulation at the time of the event.

The highly suspected coagulopathy was observed in 31 patients who were administered DP in addition to LMWH (9.45%). It was observed in nine patients who were receiving LMWH only (10.58%) (P=0.752).

In the multivariate analysis, DP addition did not have any impact on lowering the risk of demonstrated coagulopathy or highly-suspected coagulopathy.

Dipiridamol addition to LMWH did not affect the COVID-19 outcome according to our analysis. Among LMWH administered patients, the need for anticytokine treatment (P=0.163), the rate of intensive care unit need (P=0.485) and intubation (P=0.580) were similar in

Table 2. Characteristics of patients with thrombotic event

Case number	Sex	Age	Type	Dipyridamole and LMWH status at the time of thrombosis	Thrombotic event	Time from the initiation of COVID-19 related symptoms
1	M	52	Arterial	On dipyridamole and LMWH treatment dose	ST elevated MI	11 days
2	M	57	Arterial	None	ST elevated MI	2 days
3	M	66	Arterial	On dipyridamole and LMWH prophylaxis dose	Non-ST elevated MI	16 days
4	M	80	Arterial	None	Non-ST elevated MI	1 day
5	F	81	Arterial	None	Non-ST elevated MI	1 day
6	F	55	Arterial	On LMWH prophylaxis dose	Generalized arterial thrombosis of the lower extremities	3 days
7	F	40	Arterial	None	Cerebrovascular accident	5 days
8	M	43	Venous	None	DVT of the lower extremity	33 days
9	F	80	Venous	On dipyridamole and LMWH treatment dose	DVT of the upper extremity	22 days
10	F	68	Venous	On dipyridamole and LMWH prophylaxis dose	Thrombosis associated with CVC and pulmonary emboli	16 days
11	M	68	Venous	On LMWH prophylaxis dose	Cannula thrombosis of ECMO	13 days
12	M	81		On LMWH prophylaxis dose	DIC	14 days
13	F	72		On dipyridamole and LMWH prophylaxis dose	DIC	30 days
14	M	86		On dipyridamole and LMWH treatment dose	DIC	5 days
15	M	43		On dipyridamole and LMWH treatment dose	DIC	16 days

LMWH: Low-molecular weight heparin, MI: Myocardial infarction, ECMO: Extra-corporeal membrane oxygenation, CVC: Central venous catheter, DIC: Disseminated intravascular coagulopathy, DVT: Deep venous thrombosis.

between DP administered and not-administered cohorts. The non-survivor rate was 10.58% (n=9) and 6.58% (n=25) in LMWH only received group and in the DP added group, respectively (P=0.375). The time of hospitalization did not differ according to log-rank test (P=0.1422).

Discussion

Investigations strongly pointed that COVID-19 is accompanied with endothelial damage induced by activation of the coagulation cascade. Infiltration of the respiratory tissue by immune cells (neutrophils and/or macrophages and platelets) results in hypoxemia. This results in the secretion of hypoxia-inducible transcription factors and upregulated tissue factor expression. Activation of the complement system or release of pro-inflammatory cytokines, such as IL-1β and IL-6, increases the tissue damage [17-19].

Taking into consideration that COVID-19 involves primarily the endothelium, an ideal therapeutic approach would include both antithrombotic and anti-inflammatory agents. However, anticoagulation, especially with LMWH, was recommended as a potential protective approach [9], probably to prevent inflammation-mediated (micro)thrombosis [20].

DP is a safe drug. During the pandemic, it was shown that it suppressed SARS-CoV-2 replication and promoted a type I interferon (IFN) response in both in vitro and animal studies [13]. It was showed that DP supplementation significantly decreased D-dimer concentrations, increased lymphocyte and platelet recovery, and improved clinical outcomes. Another study from China suggested DP as a potent inhibitor of SARS-COV-2 main protease [21], which was not validated by the US group [22]. In our study, the definitive coagulopathy ratio was 3.5% in LMWH administered patients which did not improve with DP addition (2.13%; P=0.456). The same situation was experienced in highly suspected coagulopathy cases; the rate of thrombosis was 9.45% in the DP + LMWH group, compared to the 10.58% in the LMWH only administered group (P=0.752).

Similar to our coagulopathy outcomes, we could not detect an effect of DP on COVID-19 outcome. It is not surprising, knowing that the main pathophysiologic mechanism of COVID-19 related organ damage is via endothelial damage and associated coagulopathy development. The rate of anti-cytokine treatment, need for intensive care unit and intubation were similar in between DP administered and not-administered cohorts. The non-survivor rate difference was also not different among LMWH received group and the DP added groups.

Our institution's decision of adding DP to the COVID-19 treatment protocol was based on its

potential multifunctional effects. It has vasoprotective activity. It also has suppressive role on local fibroblasts, resulting in decreased thromboinflammation and increased local antifibrotic effect in COVID-19 [16].

Conclusion

We think that our clinical experience is valuable in showing the real-life results of DP + LMWH treatment in COVID-19. This approach did not affect the coagulopathy rate. Our data did also not document an additive effect of DP in the COVID-19 outcome. Prospective controlled trials would give more convincing results regarding the role of DP in COVID-19 endothelial dysfunction and clinical outcome.

Acknowledgements

No funding source was used, and our study was approved by the Istanbul University Istanbul Medical Faculty ethical committee (nr:27/05/2020-85499).

Disclosure of conflict of interest

None.

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