**Title:** Heparin therapy improving hypoxia in COVID-19 patients - a case series

**Authors names and ORCID:** Elnara Marcia Negri¹,² (0000-0002-6428-6066), Bruna Mamprim Piloto¹,³ (0000-0002-8756-0400), Luciana Kato Morinaga¹,³ (0000-0002-0900-2737), Carlos Viana Poyares Jardim¹,³ (0000-0003-0425-5548), Shari Anne El-Dash Lamy⁴ (0000-0003-2915-4014), Marcelo Alves Ferreira² (0000-0003-4181-760X), Elbio Antonio D'Amico⁵ (0000-0003-1069-1469), Daniel Deheinzelin¹ (0000-0002-0253-4124)

**Affiliations:**
1. Sirio-Libanes Hospital – São Paulo
2. Cell Biology Laboratory (LIM 59), Hospital das Clinicas, University of São Paulo Medical School
3. Pulmonary Department, Heart Institute, University of São Paulo Medical School
4. Critical Care Unit – A. C. Camargo Cancer Center – São Paulo
5. Hematology and Hemotherapy Department, University of São Paulo Medical School

**Corresponding author:** Elnara Marcia Negri

E-mail: emnegri@yahoo.com.br

Address: 74 Dona Adma Jafet St, 3rd floor, São Paulo, SP, Brazil

Telephone: +55(11)3514-6000
INTRODUCTION:

Since the beginning of the COVID-19 pandemic, disease severity has been linked to markers of coagulation disturbances such as prothrombin time prolongation, elevated fibrin degradation products, reduced platelet count, and especially to elevated D dimer$^{1-7}$. Higher levels of D dimer and the presence of other coagulation disturbances have been independently associated with development of respiratory failure and death in patients with COVID-19$^9$, and the use of heparin, particularly in those patients with more pronounced elevations of D dimer and in those with elevated sepsis induced coagulopathy (SIC) score, have been associated with a better prognosis$^4$ $^8$. Diabetic patients, whose levels of D dimer are greater than those of non-diabetic patients, have also been shown to have a worse prognosis regarding COVID-19$^9$. Moreover, the levels of D dimer have helped differentiate severe COVID-19 associated pneumonia from that caused by other viruses$^{10}$.

Over the past 2 months it has been consistently shown that SARS-Cov-2 causes a cytokine storm that ultimately leads to the activation of the coagulation cascade, causing thrombotic phenomena$^4$ $^8$ $^{11}$. Similarly to what happens in severe sepsis, the widespread deposition of intravascular clots compromises adequate blood supply to various organs, contributing to organ failure$^{12}$.

Disseminated intravascular coagulation (DIC) secondary to severe infection is classically associated with gram-positive or gram-negative bacteria, malaria and haemorrhagic fevers, but other viruses, such as dengue (an haemorrhagic virus), SARS-CoV and MERS-CoV, can also be responsible for systemic activation of intravascular coagulation$^{13}$ $^{14}$. 
Furthermore, in contrast to the characteristic stiffening of the lung usually seen in acute respiratory distress syndrome (ARDS), in COVID-19 patients the severe hypoxemia observed is accompanied by near normal pulmonary compliance, especially in the early stages\textsuperscript{15}. Autopsy findings from COVID-19 patients show micro thrombi in the pulmonary microvasculature\textsuperscript{16,17} (also showed by Dolhnikoff\textit{ et al}, submitted to the Journal of Thrombosis and Haemostasis, April 2020) suggesting that ventilation perfusion mismatch due to capillary obstruction could be a pivotal feature in the refractory hypoxemia presented by these patients. The anatomical distribution of this peripheral vascular bed mirrors the predominantly distal and patchy distribution of the radiological infiltrates\textsuperscript{18}.

In one of our first COVID-19 patients we noticed a concomitance of peripheral ischaemia (acro-ischemia) with the onset of respiratory distress, an observation that made us consider the hypothesis that the normal compliance respiratory failure might actually be due to extensive pulmonary capillary obstruction, and that a systemic disseminated intravascular coagulation might be playing a significant role in the hypoxemia and outcome of COVID-19 patients.

The treatment of DIC consists in slowing down the coagulation cascade by using low doses of anticoagulation, alongside vigorous specific treatment of the underlying disorder. We therefore considered adding early heparin therapy to our standard care\textsuperscript{2}. The present study is a description of the outcome of the first 27 COVID-19 patients we treated as DIC in the course of the disease.
METHODS

This study is a case series of 27 consecutive Covid-19 patients seen by our pulmonology team in Sírio Libanês Hospital – São Paulo, Brazil, between March 21st and April 12th, 2020. The study was approved by the Sírio Libanês Hospital Internal Review Board [IRB1678] which waived informed consent.

All patients with COVID-19 admitted by our team received enoxaparin 1 mg/kg SC every 24 hours (OD). Patients with a creatinine clearance under 30 mL/min received subcutaneous unfractionated heparin at a dose of 5,000 units every 8 or 6 hours. If an abrupt decrease in oxygenation or an increase in D Dimer levels was observed, enoxaparin dose was raised to 1 mg/kg SC every 12 hours (BD) and, in the event of thrombotic phenomena or worsening hypoxia, the dose was further increased to 2 mg/kg SC every 12 hours. Patients with a BMI (body mass index) of 35 or higher were also considered for the higher dose regimen. Patients in shock were treated from the beginning with intravenous heparin, targeting an APTT ratio around 1.5 to 2.0 times the normal range. If patients presented any acute thrombotic event, heparin dosing was increased to obtain an APTT approximately 2.0 to 2.5 times the normal range.

All patients received a 10-day course of azithromycin (500mg on day 1, then 250mg daily)\textsuperscript{19}. Methylprednisolone 40mg daily was initiated if a worsening in the radiological pattern accompanied by an increase in serum LDH levels was observed. If the patient presented subsequent rise in C-reactive protein, we actively searched for secondary infection and promptly initiated antibiotics. Only two of our patients used hydroxychloroquine before receiving anticoagulation therapy.
RESULTS

We followed a total of 27 hospitalized patients with a diagnosis of Covid-19, all confirmed by PCR. Seventy percent were male, their mean age was 56 ± 17 years, mean BMI was 28.8 ± 6 kg/m², and comorbidities were present in 56% of them (Table 1). The mean WHO classification score³ at admittance was 4.0 ± 1.2 (Figure 1). Entry CT scans showed radiologic infiltrates compromising up to 25% of lung area in 22% of patients, 25-50% of lung area in 48% of patients, and 30% of patients presented infiltrates in over half of lung parenchyma. Symptoms started an average of 9.6 ± 4.0 days prior to hospitalization, and the anticoagulation protocol was initiated an average of 3.4 ± 4.0 days after admission. The average level of D Dimer during the follow up period was 1,637± 1,967 ng/mL FEU, with a peak value of 3,544 ± 5,914 ng/mL FEU. Only 5 patients (19%) never had a D dimer level above 500 ng/mL FEU.

Of the 27 consecutive patients, 15 (56%) were discharged from hospital after an average 7.3 (± 4.0) days. One patient was transferred to another hospital on the 4th day and lost follow-up. Nine patients (33%) were admitted to ICU, 3 (33%) of which have already been discharged to the ward after an average 9.3(±4.5) days. Eight patients (30%) required intubation, half of which (4 patients) have already been successfully extubated after an average 10.3(± 1.5) days of mechanical ventilation, while the other half (4 patients) are currently being weaned from the ventilator, and 2 of them have required a tracheostomy.

Interestingly enough, rotational thromboelastometry (ROTEM) performed in four patients, showed an increase in α-angle, amplitude 10 minutes after clotting time (A10) and maximum clot firmness (MCF) pointing to a persistent hypercoagulability state, despite their ongoing heparin use.
Figure 2 depicts the gradual improvement in PaO₂/FiO₂ ratio along the first 72 hours in relation to pre-anticoagulation values. Analysis was conducted for the whole series (A) and considering only patients with moderate to severe disease (B) according to the WHO score (p<0.02 for both groups). For non-mechanically ventilated patients PaO₂/FiO₂ ratio was calculated according to the mask or nasal catheter oxygen flow and oxymetry²⁰.

We observed no deaths or haemorrhagic complications due to anticoagulation during the study period.
DISCUSSION

Our results suggest the important role of disseminated intravascular coagulation as one of the main mechanisms of organ failure in COVID-19 and the potential response to early anticoagulation therapy.

The significant improvement observed in oxygen exchange and clinical symptoms observed in these COVID19 patients in response to the anticoagulation points to potential role for systematic use of heparin in the treatment of such patients. The high incidence of thrombotic events that has been reported in COVID-19 patients\(^{21}\), as well as the fact that similar observations were reported in the other recent coronavirus outbreaks\(^{13,14}\), further corroborate this line of reasoning. This is not surprising, as severe cases of COVID19 meet the laboratory criteria of DIC\(^4\) of thrombotic pattern, in which fibrinogen does not drop and prothrombotic phenomena override the haemorrhagic ones\(^{22}\). The histological findings of thrombi in the microvasculature documented in the autopsies of COVID-19 patients\(^{17}\), further support the belief that in COVID-19 we are facing a thrombotic organ-failure type of DIC that should be treated with heparin\(^{22,23}\). This might explain the previous findings of an association between heparin use during hospital stay and a reduced mortality\(^4\).

Thromboelastography showing a pattern of hypercoagulability despite the use of heparin during the course of viral diseases has been previously reported\(^{24}\). In fact, many viruses known to induce a state of hypercoagulability\(^{25}\) have a similar pattern of disease, including the timeframe of clinical manifestations\(^{26}\), suggesting a common pattern of response.

Although the principle that early anticoagulation therapy for sepsis probably causes uncontrolled immunothrombosis and pathological systemic DIC, since the
The presence of neutrophil extracellular traps (NETs) and hypercoagulation in DIC localize the infection, this is well described for bacteria but not for virus, where virus-induced NETs could be a pathogenic mediator.

The PaO₂/FiO₂ ratio improvement observed in our patients after starting heparin is in agreement with the idea of a significant perfusion component explaining the mechanism of respiratory failure with the distinct pattern of marked hypoxia and preserved lung compliance that characterizes severe COVID19 patients. It has been argued that this could be secondary to the loss of lung perfusion regulation and hypoxic vasoconstriction, but the clinical response to heparin rather suggests hypoxia due to extensive clogging of pulmonary microcirculation. Interestingly, the use of tissue Plasminogen Activator (tPA) has been shown to promote a non-sustained elevation of PaO₂/FiO₂ ratio. In our opinion, given the marked hypercoagulability seen in these patients - and again in accordance with the autopsy findings - judicious tailoring of heparin doses is needed to prevent capillary reocclusion while avoiding the risks of bleeding complications.

The fact that this is a retrospective study without a control arm does not yet allow us to definitively conclude that heparin in tailored doses should be systematically employed in all COVID19 patients. Nonetheless, our findings in this early group of patients certainly provide food for thought and perhaps a rationale to justify using a readily available and well-known drug such as heparin to ameliorate the dim prognosis of such sick patients while we await the more solid data on this subject that could be provided by a prospective controlled study.
REFERENCES

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA* 2020 doi: 10.1001/jama.2020.1585 [published Online First: 2020/02/08]

2. Chen G, Wu D, Guo W, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest* 2020 doi: 10.1172/JCI137244 [published Online First: 2020/03/29]

3. Zhang JJ, Dong X, Cao YY, et al. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy* 2020 doi: 10.1111/all.14238 [published Online First: 2020/02/23]

4. Tang N, Bai H, Chen X, et al. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. *J Thromb Haemost* 2020 doi: 10.1111/jth.14817 [published Online First: 2020/03/29]

5. Han H, Yang L, Liu R, et al. Prominent changes in blood coagulation of patients with SARS-CoV-2 infection. *Clin Chem Lab Med* 2020 doi: 10.1515/cclm-2020-0188 [published Online First: 2020/03/17]

6. Chen T, Wu D, Chen H, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. *BMJ* 2020;368:m1091. doi: 10.1136/bmj.m1091 [published Online First: 2020/03/29]

7. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020;395(10229):1054-62. doi: 10.1016/S0140-6736(20)30566-3 [published Online First: 2020/03/15]

8. Wu C, Chen X, Cai Y, et al. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. *JAMA Intern Med* 2020 doi: 10.1001/jamainternmed.2020.0994 [published Online First: 2020/03/14]

9. Guo W, Li M, Dong Y, et al. Diabetes is a risk factor for the progression and prognosis of COVID-19. *Diabetes Metab Res Rev* 2020:e3319. doi: 10.1002/dmrr.3319 [published Online First: 2020/04/02]

10. Yin S, Huang M, Li D, et al. Difference of coagulation features between severe pneumonia induced by SARS-CoV2 and non-SARS-CoV2. *J Thromb Thrombolysis* 2020 doi: 10.1007/s11239-020-02105-8 [published Online First: 2020/04/05]
11. Mehta P, McAuley DF, Brown M, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 2020;395(10229):1033-34. doi: 10.1016/S0140-6736(20)30628-0 [published Online First: 2020/03/21]

12. Burzynski LC, Humphry M, Pyrillou K, et al. The Coagulation and Immune Systems Are Directly Linked through the Activation of Interleukin-1alpha by Thrombin. *Immunity* 2019;50(4):1033-42 e6. doi: 10.1016/j.immuni.2019.03.003 [published Online First: 2019/03/31]

13. de Wit E, van Doremalen N, Falzarano D, et al. SARS and MERS: recent insights into emerging coronaviruses. *Nat Rev Microbiol* 2016;14(8):523-34. doi: 10.1038/nrmicro.2016.81 [published Online First: 2016/06/28]

14. Giannis D, Ziogas IA, Gianni P. Coagulation disorders in coronavirus infected patients: COVID-19, SARS-CoV-1, MERS-CoV and lessons from the past. *Journal of Clinical Virology* 2020;104362 @ 1386-6532.

15. Gattinoni L, Coppola S, Cressoni M, et al. Covid-19 Does Not Lead to a "Typical" Acute Respiratory Distress Syndrome. *Am J Respir Crit Care Med* 2020 doi: 10.1164/rccm.202003-0817LE [published Online First: 2020/04/02]

16. Tian S, Hu W, Niu L, et al. Pulmonary Pathology of Early-Phase 2019 Novel Coronavirus (COVID-19) Pneumonia in Two Patients With Lung Cancer. *J Thorac Oncol* 2020 doi: 10.1016/j.jtho.2020.02.010 [published Online First: 2020/03/03]

17. Yao XH, Li TY, He ZC, et al. [A pathological report of three COVID-19 cases by minimally invasive autopsies]. *Zhonghua Bing Li Xue Za Zhi* 2020;49(0):E009. doi: 10.3760/cma.j.cn112151-20200312-00193 [published Online First: 2020/03/17]

18. Ye Z, Zhang Y, Wang Y, et al. Chest CT manifestations of new coronavirus disease 2019 (COVID-19): a pictorial review. *Eur Radiol* 2020 doi: 10.1007/s00330-020-06801-0 [published Online First: 2020/03/21]

19. Cramer CL, Patterson A, Alchakaki A, et al. Immunomodulatory indications of azithromycin in respiratory disease: a concise review for the clinician. *Postgrad Med* 2017;129(5):493-99. doi: 10.1080/00325481.2017.1285677 [published Online First: 2017/01/25]

20. Lobete C, Medina A, Rey C, et al. Correlation of oxygen saturation as measured by pulse oximetry/fraction of inspired oxygen ratio with Pao2/fraction of inspired oxygen ratio in a heterogeneous sample of critically ill children. *J Crit Care* 2013;28(4):538 e1-7. doi: 10.1016/j.jcrc.2012.12.006 [published Online First: 2013/02/12]

21. Klok F, Kuip M, van der Meer N, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb Res* doi: 10.1016/j.thromres.2020.04.013
22. Wada H, Matsumoto T, Yamashita Y, et al. Disseminated intravascular coagulation: testing and diagnosis. *Clin Chim Acta* 2014;436:130-4. doi: 10.1016/j.cca.2014.04.020 [published Online First: 2014/05/06]

23. Wada H, Thachil J, Di Nisio M, et al. Guidance for diagnosis and treatment of DIC from harmonization of the recommendations from three guidelines. *J Thromb Haemost* 2013 doi: 10.1111/jth.12155 [published Online First: 2013/02/06]

24. Wilson AJ, Martin DS, Maddox V, et al. Thromboelastography in the Management of Coagulopathy Associated With Ebola Virus Disease. *Clin Infect Dis* 2016;62(5):610-12. doi: 10.1093/cid/civ977 [published Online First: 2015/11/28]

25. Subramaniam S, Scharrer I. Procoagulant activity during viral infections. *Front Biosci (Landmark Ed)* 2018;23:1060-81. doi: 10.2741/4633 [published Online First: 2017/09/21]

26. Gai ZT, Zhang Y, Liang MF, et al. Clinical progress and risk factors for death in severe fever with thrombocytopenia syndrome patients. *J Infect Dis* 2012;206(7):1095-102. doi: 10.1093/infdis/jis472 [published Online First: 2012/08/02]

27. Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil extracellular traps kill bacteria. *Science* 2004;303(5663):1532-35 % @ 0036-8075.

28. Wang J, Hajizadeh N, Moore EE, et al. Tissue Plasminogen Activator (tPA) Treatment for COVID-19 Associated Acute Respiratory Distress Syndrome (ARDS): A Case Series. *J Thromb Haemost* 2020 doi: 10.1111/jth.14828 [published Online First: 2020/04/09]
FIGURE SUBTITLES

Table 1 - Comorbidities incidences

Figure 1 - WHO score at admission of all patients

Figure 2 - PO₂/FiO₂ ratio over time from start of anticoagulation.
| Comorbidities                  | n  | %    |
|-------------------------------|----|------|
| Total                         | 15 | 56%  |
| Diabetes                      | 3  | 11%  |
| Hypertension                  | 7  | 26%  |
| Heart disease                 | 3  | 11%  |
| Previous lung disease         | 2  | 7%   |
| Cancer                        | 1  | 4%   |
| Other                         | 7  | 26%  |
WHO score at admission

- **Discharged**
- **In hospital**

Patients (n)

WHO score

- 0
- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
A. \( \text{PaO}_2 / \text{FiO}_2 \) ratio after heparin treatment (all patients)

B. \( \text{PaO}_2 / \text{FiO}_2 \) ratio after heparin treatment (WHO score \( \geq 4 \))

\[ p = 0.013 \]

\[ p = 0.015 \]