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# EFFECT OF LOW OR HIGH DOSES OF LOW MOLECULAR WEIGHT HEPARIN ON THROMBIN GENERATION AND OTHER HEMOSTASIS PARAMETERS IN COVID-19 CRITICAL PATIENTS

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Authorship

AC, FR, FMP, FP conceived the study, designed the study.

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The clinical picture of the coronavirus 2 (SARS-CoV-2)/COVID-19-related acute respiratory syndrome is often associated with a coagulopathy.[1] An elevated D-dimer has been linked with an unfavorable prognosis in COVID-19 patients. In a recent cohort study, 71% of patients who died matched the International Society of Thrombosis and Hemostasis (ISTH) criteria for disseminated intravascular coagulation (DIC)[2], while this percentage was only 0.6% in patients who survived.[3] In two recent studies, the authors described the presence of a hypercoagulability state in COVID-19 affected patients, in the absence however of pathognomonic signs of DIC.[4,5] Thus, the nature of this coagulopathy is not fully understood. Clinical evidences suggest that this COVID-19-related

coagulopathy is associated with an increased risk of both venous and arterial thrombotic events.[6,7] The management of these thromboembolic complications is based on the use of heparin in the absence of contraindications (active bleeding and a platelet count <30x10$^9$/l).[8] However, the efficacy of heparin remains to be validated. The benefit/risk of using heparin, as well as the timing of starting anticoagulants and at which dose, is controversial.[9,10] We carried out an observational study to investigate different coagulation parameters, in particular thrombin generation assay (TGA), in COVID-19 critical patients and to correlate these results with different doses of low molecular weight heparin (LMWH) administered to these subjects. The study was approved by the Ethics Committee of the Sapienza University of Rome (n. 109/2020). The TGA is a global coagulation test that provides a direct assessment of plasma coagulability.[11] The following TGA parameters were evaluated: T-Lag, time that follows the addition of the trigger until the initiation of thrombin generation; time to peak (tt-Peak), time to the highest thrombin concentration; thrombin peak (Peak), the highest thrombin concentration; endogenous thrombin potential (ETP), area under the curve, total amount of thrombin generation. Between April and May 2020, a consecutive series of 27 COVID-19 patients admitted to the Intensive Care Unit (ICU) of the Sapienza University Hospital in Rome were included in the study. Seventeen (63%) were males and 10 (37%) females. Mean age was 66 years (range 38-85). At the time of the sample collection, patients were swab culture positive for COVID-19, were affected by acute respiratory failure without an active and diagnosed thromboembolic event. All patients were intubated and mechanically ventilated. Fourteen patients (51.9%) were treated with low-dose LMWH (100 IU/kg/day) and 13 (48.1 %) with high-dose LMWH (100 IU/kg/twice daily). After the initial administration of LMWH at a dose of 100 IU/Kg, based on the growing evidence of potential thrombotic events we decided to increase the prophylactic dose up to 100 IU/kg twice daily.[12] This is why we observed two differently treated populations. The median time from the first LMWH administration and the blood sample collection was 5 days (range 2-21); laboratory assays were evaluated on a single occasion and all blood samples were collected just before starting the LMWH administration. We observed an increase of the mean d-Dimer values to 4686.07 ng/ml (n.v. <550 ng/ml), as well as of FVIII, vWF:Ag and vW:RCo: 209% (n.v. 58-130%), 319.54% (n.v. 50-160%) and 310.60% (n.v. 52-124%), respectively (Table 1). Increased levels of FVIII are a potent sign of hypercoagulability and increased levels of vWF:Ag and vWF:RCo are indicative of an

endothelial derangement. These altered coagulation parameters observed in COVID-19 patients could be related to an active response due to a marked alveolar inflammatory cell infiltrate with a consequent systemic cytokine storm. On the contrary, we found laboratory parameters compatible with a diagnosis of DIC only in few patients: a prolonged PT was observed in 29.6% of cases, a prolonged aPTT in 14.8%, a reduced fibrinogen in 29.6%, a reduced AT in 37% and a decreased platelet count in 7.4% (Table 1). We cannot exclude that these results may be associated with the heparin administration which was ongoing in all patients from at least 2 days prior to the blood collection. With regard to TGA, we observed an overall increased mean values of the T-Lag (7.70 min; n.v. <4.3 min), Peak (122.22 nM; n.v. <106.2 nM) and tt-Peak (13.38 min; n.v. <9.8 min); the mean ETP was within the normal range (953.51 nM min; n.v. <984.12 nM min). All parameters were influenced by LMWH administration in a dose-dependent manner. In fact, we observed an increased mean value of the T-Lag and tt-Peak, and a decrease of the mean value of Peak and ETP in the group of patients on high-dose LMWH compared to the data observed in patients on low-dose LMWH (Table 1, Figure 1). ETP was the only parameter that resulted significantly increased (p=0.046), probably because ETP is more influenced by LMWH doses. A thrombotic complication occurred in 3/27 patients (11%): 2 pulmonary embolisms and 1 acute myocardial ischemia (MI). All 3 patients were on low-dose LMWH. The patient with the MI was previously on high-dose LMWH, but because of a tracheostomy site bleeding he was switched to the low-dose regimen: three days later the patient experienced the MI. The low rate of thrombo-embolic complications observed in our patients is probably due to the fact that all patients were put on LMWH prophylaxis as soon as they arrived in the ICU and that half of them were on high dose LMWH. Moreover, in our COVID-19 patients the use of heparin did not result in a higher risk of bleeding complications: we observed only 1 hemorrhagic event. In conclusion, our results suggest that COVID-19 critically ill patients develop a state of hypercoagulability that is present also during the administration of LMWH. Different doses of LMWH have an influence on the laboratory results, especially with regard to the total amount of thrombin generation, with a significant reduction only in patients receiving high-dose heparin. In our experience, the use of a higher dose of LMWH as thromboembolic prophylaxis reduced the incidence of thrombotic complications without an increase in bleeding events. Randomized clinical trials are required to conclusively define the efficacy and safety of different doses of LMWH in patients

with severe COVID-19 infection. Moreover, further studies on the utility of changes in thrombin generation parameters in predicting risk of thromboembolism in severe COVID-19 affected patients are required.

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**Table 1.** Coagulation parameters in the 27 COVID-19 patients (mean values)

| **Coagulative tests (normal range)** | **Mean value (min-max) (n=27)** | **HD patients Mean value (n=14)** | **LD patients Mean value (n=13)** | **P value (HD versus LD)** |
|---|---|---|---|---|
| **PT ratio** (0.92-1.18) | 1.12 (0.9-1.64) | 1.18 | 1.06 | 0.11 |
| **aPTT ratio** (0.81-1.20) | 1.14 (0.81-3.06) | 1.21 | 1.07 | 0.31 |
| **Fibrinogen** (196-440 mg/dL) | 383.44 (62-663) | 365 | 404 | 0.9 |
| **AT** (80-120%) | 88.88 (67.7-120) | 90 | 87 | 0.64 |
| **D-dimer** (<550 ng/mL) | **4686.07** (465-35782) | 4409 | 4985 | 0.48 |
| **Platelets** (100-450 x $10^9$/l) | 214 (51-378) | 213 | 216 | 0.9 |
| **FVIII** (58-130%) | **209.07** (108.6-392.9) | 214 | 203 | 0.66 |
| **VWF:Ag** (50-160%) | **319.54** (158.4-557.1) | 352 | 284 | 0.1 |
| **VWF:Rco** (52-124%) | **310.60** (143.5-600.6) | 357 | 261 | 0.12 |

| | | | | |
|---|---|---|---|---|
| **PC** (70-115%) | 114.35 (72.5-149.8) | 114 | 115 | 0.96 |
| **PS** (64-124%) | 71.14 (35.9-98.2) | 73 | 69 | 0.61 |
| **T-Lag** (≤4.3 min) | **7.70** (3-32.17) | 8.9 | 6.5 | 0.32 |
| **tt-Peak** (≤9.8 min) | **13.38** (5.17-49.67) | 15.4 | 11.2 | 0.81 |
| **Peak** (≤106.2 nM) | **122.22** (5.31-268.48) | 98.1 | 148.4 | 0.69 |
| **ETP** (≤984.12 nM min) | **953.51** (1-2357.21) | **705.19** | **1222.52** | **0.01** |

**Fig. 1.** Thrombin generation curves in 2 single patients (one patient on high and one on low dose of LMWH) and normal plasma control.