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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig S1. Geographical distribution of participants.

Table S1. List of the GIMEMA clinical trials included in the study.

Table S2. List of principal investigators (PIs) participating in the survey.

Table S3. Survey questions and results.

Data S1. Supplementary methods.

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Effect of low or high doses of low-molecular-weight heparin on thrombin generation and other haemostasis parameters in critically ill patients with COVID-19

The clinical picture of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)/coronavirus disease 2019 (COVID-19)-related acute respiratory syndrome is often associated with a coagulopathy.[1] An elevated D-dimer has been linked with an unfavourable prognosis in patients with COVID-19. 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 evidence suggests 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 of $<30 \times 10^9$/l).[8] However, the efficacy of heparin remains to be validated. The benefit/risk of using heparin, as well as the timing of starting anti-coagulants and at which dose, is controversial.[9,10] We carried out an observational study to investigate different coagulation parameters, in particular the thrombin generation assay (TGA), in critically ill patients with COVID-19 and to correlate these results with different doses of low-molecular-weight heparin (LMWH) administered to these patients. The study was approved by the Ethics Committee of the Sapienza University of Rome (no. 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 patients with COVID-19 admitted to the Intensive Care Unit (ICU) of the Sapienza University Hospital in Rome were included in the study; 17 (63%) were males and 10 (37%) were females. The mean (range) age was 66 (38–85) years. 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. In all, 14 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 (range) time from the first LMWH administration and the blood sample collection was 5 (2–21) days; laboratory assays were evaluated on a single occasion and all blood samples were collected just before starting the LMWH administration. We observed an increase in the mean D-dimer values to 4686·07 ng/ml [normal value (n.v.) <550 ng/ml], as well as of factor VIII (FVIII), von Willebrand factor antigen level (VWF:Ag) and VWF ristocetin co-factor (VWF:RCo): 209% (n.v. 58–130%), 319·54% (n.v. 50–160%) and 310·60% (n.v. 52–124%) respectively (Table I). 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 patients with COVID-19 could be related to an active response due to a marked alveolar

Table I. Coagulation parameters in the 27 patients with COVID-19 (mean values).

Coagulative tests (normal range)	Mean value (range) (n = 27)	HD patients, mean value (n = 14)	LD patients, mean value (n = 13)	P (HD vs. 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, mg/l (196–440)	383·44 (62–663)	365	404	0·9
AT, % (80–120)	88·88 (67·7–120)	90	87	0·64
D-dimer, ng/ml (<5500)	**4686·07** (465–35 782)	4409	4985	0·48
Platelets, $\times 10^9$/l (100–450)	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, min (≤4·3)	**7·70** (3–32·17)	8·9	6·5	0·32
tt-Peak, min (≤9·8)	**13·38** (5·17–49·67)	15·4	11·2	0·81
Peak, nM (≤106·2)	**122·22** (5·31–268·48)	98·1	148·4	0·69
ETP, nM min (≤984·12)	**953·51** (1–2357·21)	**705·19**	**1222·52**	**0·01**

aPTT, activated partial thromboplastin time; AT, antithrombin; ETP, endogenous thrombin potential; FVIII, factor VIII; HD, high-dose LMWH; LD, Low-dose LMWH; PC, protein C; Peak, thrombin peak (highest thrombin concentration); PS, protein S; PT, prothrombin time; T-Lag, time that follows the addition of the trigger until the initiation of thrombin generation; tt-Peak, time to the highest thrombin concentration; VWF:Ag, von Willebrand factor antigen level; VWF:RCo, VWF ristocetin co-factor.
The bolded values are the abnormal findings of our study.

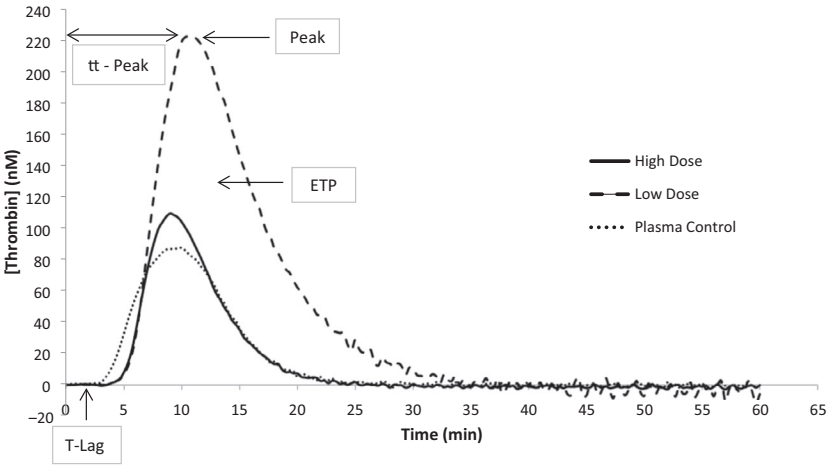

Fig. 1. Thrombin generation curves in two patients (one patient on high- and one on low-dose LMWH) and normal plasma control.

inflammatory cell infiltrate with a consequent systemic cytokine storm. On the contrary, we found laboratory parameters compatible with a diagnosis of DIC only in a few patients: a prolonged prothrombin time (PT) was observed in 29·6% of patients, a prolonged activated partial thromboplastin time (aPTT) in 14·8%, a reduced fibrinogen in 29·6%, a reduced antithrombin (AT) in 37% and a decreased platelet count in 7·4% (Table I). 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 the TGA, we observed 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 in 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 I, Fig 1). ETP was the only parameter that was significantly increased ($P = 0{\cdot}046$), probably because ETP is influenced more by LMWH dosing. A thrombotic complication occurred in three of the 27 patients (11%): two pulmonary embolisms and one acute myocardial ischaemia (MI). All three patients were on low-dose LMWH. The patient with the MI was previously on high-dose LMWH, but because of a tracheostomy site bleed, he was switched to the low-dose regimen: 3 days later the patient experienced the MI. The low rate of thromboembolic complications observed in our present patients is probably due to the fact that all patients were given LMWH prophylaxis as soon as they arrived in the ICU and that half of them were on high-dose LMWH. Moreover, in our present patients with COVID-19 the use of heparin did not result in a higher risk of bleeding complications: we observed only one haemorrhagic event. In conclusion, our present results suggest that critically ill patients with COVID-19 develop a state of hypercoagulability that is also present during the administration of LMWH. Different doses of LMWH have an influence on the laboratory results, especially for 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. Randomised 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 patients severely affected with COVID-19 are required.

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Conflict of interest

The authors have no competing interests.

Author contributions

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Supporting Information

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Table SI. Coagulation parameters in the 27 patients with COVID-19 (median values).

Data S1. Supplementary methods.

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COVID-19 comorbidities, associated procoagulant extracellular vesicles and venous thromboembolisms: a possible link with ethnicity?

In view of numerous recent reports describing increased risks of thrombosis in coronavirus disease 2019 (COVID-19) amongst certain ethnicities in which co-morbidities are more prevalent, besides the identified risk factors, procoagulant plasma extracellular vesicles have not been considered. Several comorbidities predict mortality in patients with COVID-19, some of which are more prevalent in Black, Asian and Minority Ethnic (BAME) groups. In a meta-analysis of seven studies (1,576 infected patients) the co-morbidities included hypertension (21·1%), diabetes (9·7%), cardiovascular disease (CVD), (8·4%) and respiratory system disease (1·5%).[1] Of the critically ill COVID-19 patients with these co-morbidities and an associated hyperinflammatory state admitted to intensive care units (ICUs), up to 31% suffered thrombotic episodes (even with thromboprophylaxis), in particular, venous thromboembolisms (VTE),[2] one of the important sequelae of COVID-19.

Extracellular vesicles (EVs) are nano-sized, membrane-bound vesicles released from cells that carry nucleic acids and proteins, and which mediate intercellular communication. In COVID-19-associated co-morbidities, including diabetes, CVD and risk factors such as hypertension, elevated angiotensin II (Ang II) and obesity, levels of circulating EVs are raised.[3-6] Increased EVs, especially from injured or TNF-α-stimulated endothelial cells (EC) carrying tissue factor (TF^+), or from platelets exposing phosphatidylserine (PS^+), are known to be procoagulant and to cause VTE.[7] Levels of EVs also correlate with von Willebrand Factor (vWF), a marker of EC damage and dysfunction.[8] This endothelial injury is due to Ang II-mediated superoxide damage and hypoxia-mediated oxidative stress as well as severe acute respiratory syndrome coronavirus (SARS-CoV-2) binding to angiotensin-converting enzyme 2 (ACE2) on ECs and ensuing complement-mediated inflammation[9] (Fig 1A,B). Previously, endothelial cell-derived medium EV (EC-mEV) levels were found to be associated with stroke.[10] More recently, $CD31^+$ and $CD144^+$ EC-derived mEVs, phenotypes reflecting apoptosis and structurally damaged endothelium, were found to relate to risk factors for CVD and, thus, increased the risk of VTE, especially raised triglycerides, hypertension and metabolic syndrome.[4]

Could it therefore, be that raised circulating EVs with modulated cargo in certain comorbidity groups, in addition to infection with SARS-CoV-2, renders COVID-19 patients particularly susceptible to VTE? If so, EVs could be used as biomarkers in COVID-19. A TF^+-EV procoagulant activity assay, for example, based on fibrin formation, previously used in monitoring the risk of VTE in cancer patients, should be tried in COVID-19.