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Thrombosis Update

Intervention in COVID-19 linked hypercoaguable states characterized by circuit thrombosis utilizing a direct thrombin inhibitor

ARTICLE INFO

Progressive respiratory failure and acute kidney injury (AKI) are associated with the highest risk of mortality in SARS-CoV-2-associated Coronavirus disease 2019 (COVID-19) [1–3]. These manifestations are linked to a systemic microvascular thrombosis associated with persistent activation of the complement [4] and coagulation [5,6] cascades. We investigated five individuals with critical COVID-19, including respiratory failure and an AKI complicated by circuit thrombosis during continuous renal replacement therapy (CRRT), who were hospitalized in an intensive care unit (ICU) setting. Thrombosis occurred despite use of standard ICU anti-coagulation regimens [6,7]. This is a frequent complication of COVID-19, noted in 28 of 29 cases in one series of CRRT cases [8]. Appropriate interventions are unknown. Circuit thrombosis was heralded by a marked rise in D-dimer levels in 4 of our 5 cases, and a persistent elevation of fibrinogen in all cases. Although fibrinogen is not an independent risk factor for COVID-19 mortality, its elevation is relevant to CRRT clotting for two reasons: high levels of fibrinogen are associated with heparin resistance [5], and ultrafiltration results in concentration of fibrinogen on dialyzer capillaries [8]. Resolution of the need for repetitive filter replacement and a decline in D-dimer and fibrinogen levels followed substitution of argatroban, a direct thrombin inhibitor here prescribed as an "off-label" medication (see Fig. 1).

All five patients had co-morbidities classic for severe/critical COVID-19 as well as elevated lactate dehydrogenase (LDH) and absolute neutrophil counts (ANC) (Table 1). Measures of organ and tissue dysfunction consistent with a hypercoaguable state, including liver function abnormalities and skeletal muscle damage, assessed by aspartate transaminase (AST) and creatine kinase (CK), respectively, were also prevalent (Table 1). COVID-19-associated coagulopathies have been shown to correlate with pro-inflammatory cytokines interleukin (IL)-6 and C-reactive protein (CRP) [5]. CRP levels were high in all 5 cases, and IL-6 levels elevated in 3 of the 4 cases tested (Table 1).

No patients had evidence of disseminated intravascular coagulation (DIC) by International Society on Thrombosis and Hemostasis diagnostic criteria, SOFA (sequential organ system failure assessment) scoring, or elevation of the International Normalized Ratio in the setting of thrombocytopenia (Table 1). Although 4T scores to evaluate the possibility of a heparin-induced thrombocytopenia (HIT) were ≤4 in all cases, indicating a low probability of this phenomenon, ELISAs for anti-platelet factor 4

(PF4) antibodies were performed in Cases 2, 3, and 5 and were negative.

Case 1. Rapid escalation of D-dimers to >16,000ng/ml (normal 0–229ng/ml) occurred despite use of subcutaneous unfractionated heparin (UFH; 5000 units twice daily) followed by intermediate dose enoxaparin, 0.5mg/kg body weight every 12 hours, targeting peak anti-Xa levels of 0.4–0.5 IU/ml. Persistent filter clotting was noted on institution of CRRT, with complete resolution following argatroban initiation ((2 μg/kg/min as a continuous intravenous infusion, targeting an activated partial thromboplastin time (aPTT) ≥2.5 times baseline). This was accompanied by normalization of fibrinogen (Table 1), and a 60% decrease in D-dimers, though persisting >7000ng/ml (Figure).

Case 2. A marked, progressive increase in D-dimers was noted on therapeutic intravenous UFH, targeting anti-Xa levels of 0.4–0.7 IU/ml, correlating with aPTT times of >80 seconds (normal 27.6–36.6 seconds). Persistent filter clotting occurred on institution of CRRT, with complete resolution following argatroban substitution. Fibrinogen levels declined from a peak of 585ng/ml (normal 180–400ng/ml) at the initiation of argatroban to normal levels within 3 days (Table), despite the fact that D-dimers, though declining, remained elevated (Figure). Successful transition back to therapeutic UFH was achieved five days later.

Case 3. A steady decline in D-dimers paralleled initiation of therapeutic UFH, with aPTT times >80 seconds (Figure). Despite this salutary trend, fibrinogen levels remained elevated at ≥550mg/dL, and circuit clotting was observed on initiation of CRRT (Figure). Anti-thrombin (AT)III levels, drawn on UFH, were suppressed at 58% (normal 82–136%). With persistent filter clotting over the next four days, UFH was replaced by argatroban on day four. This led to resolution of filter clotting, with successful transition back to prophylactic UFH eight days later. Cessation of circuit clotting was accompanied by normalization of fibrinogen (Table 1), albeit D-dimers remained ~2500ng/ml (Figure).

Case 4. A continued rise in D-dimers occurred despite use of prophylactic subcutaneous UFH. CRRT was initiated and persistent circuit thrombosis, requiring multiple filter flushes and replacement, was noted over the ensuing four days. With a marked peak in D-dimers on day 4 of CRRT use, argatroban was substituted. ATIII levels, drawn in the absence of heparin, were within normal limits (92%). Argatroban led to
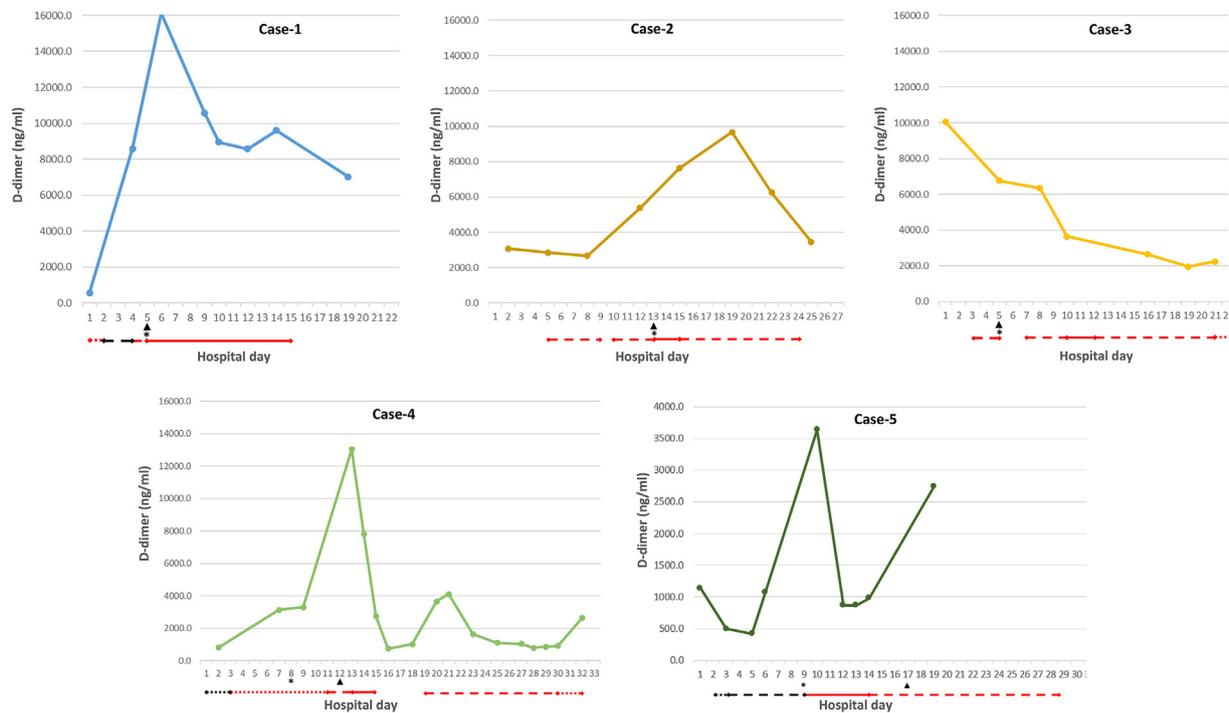
Fig. 1. Intervention with a direct thrombin inhibitor in persistent circuit clotting occurring in COVID-19 patients on CRRT. Five patients with SARS-CoV-2 infection and critical disease, involving respiratory failure and acute kidney injury, were followed from the time of ICU admission (hospital day 1). Day of institution of continuous renal replacement therapy (CRRT) is marked by an asterisk. Day of first recognition of a circuit clot, which persisted after dialysis membrane flushes, is marked by an arrow. Anticoagulation regimens are indicated as: argatroban, red solid line; therapeutic intravenous heparin, red dashed line; prophylactic enoxaparin (0.5mg/kg body weight, once daily), black dotted line; intermediate dose enoxaparin (0.5mg/kg body weight twice daily), black dashed line; therapeutic dose enoxaparin (1mg/kg body weight twice daily), black solid line. The gap in anticoagulation for Case 4 on days 15–17 related to concern for a bleed following an acute drop in hemoglobin. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Clinical and laboratory data[a].

Case	Age	Obesity	Co-morbidity	ANC (x10³)	LDH (U/L)	Platelets (x10⁹/L)	INR	CRP (mg/dL)/IL-6 (pg/ml)	Fibrinogen (mg/dL) Pre- Post-argatroban		Ferritin (ng/mL)	CK (U/L)	AST/ALT (U/L)
1	77	Overwt.	HTN	13.3	1037	183	1.2	>30.4/ND	815	392	2793	2130	461/258
2	55	Overwt.	Renal transplant, a. fib.	14.8	456	114	1.2	24.4/125	585	146	8699	64	102/36
3	56	No	DM, CKD	25.9	2254	107	1.2	22.6/25	586	242	4713	>7800	1027/538
4	65	Overwt.	HTN, HLD	22.1	772	232	1.0	15.2/<5	855	397	2020	87	540/181
5	65	No	HTN, HLD, DM2	14.9	847	152	1.2	26.2/18	672	ND	2564	1551	151/112

[a] Laboratory values represent peak values for the period preceding recognition of CCRT circuit thrombosis. Obesity: overweight, body mass index (BMI) > 25 but <30. HTN, hypertension. HLD, hyperlipidemia. DM2, type 2 diabetes. A. fib., atrial fibrillation. CKD, chronic kidney disease. ANC, absolute neutrophil count. LDH, lactate dehydrogenase (nl. 118–230 U/L). Plts, platelet count. INR, International Normalized Ratio. CRP, C-reactive protein (nl ≤ 0.9mg/dL). IL-6, interleukin-6 (nl ≤ 5pg/ml). CR, creatine kinase (nl. 34–145 U/L). AST, aspartate transaminase (nl. ≤34U/L). ALT, alanine transferase (nl. 10–49U/L). Ferritin, nl.10–291ng/ml. Fibrinogen, nl.180–400 mg/dL.

resolution of filter clotting and, over the next three days, normalization of fibrinogen and a decrease in D-dimers, though persisting at ~1000ng/ml (Figure). Successful transition to therapeutic UFH occurred four days later.

Case 5. Rising D-dimers were noted on intermediate dose enoxaparin. Argatroban was substituted upon detection of a spike in D-dimers on the day CRRT was started, leading to a marked decline in D-dimers (Figure), normal fibrinogen levels (data not shown), and no detectable circuit thrombi or other clotting issues over the subsequent 7 days. Argatroban was then discontinued in favor of therapeutic UFH, with maintenance of aPTT levels >80 seconds. Over the next four days, fibrinogen levels increased to 672mg/dL (Table) in the context

of a resurgence of D-dimers (Figure), and circuit clotting and arterial thrombi were noted 4 days later.

The development of persistent circuit thrombosis during CRRT in the context of standard anticoagulation regimens, observed in our five cases, and by others for both CCRT and extracorporeal membrane oxygenation (ECMO), greatly exceed rates typical in the ICU setting [5,8]. This is another example of the fact that resistance to UFH, as well as sub-optimal peak anti-Xa levels following therapeutic low molecular weight heparin (LMWH), appears to be common among COVID-19 patients in the ICU [9]. A direct thrombin inhibitor such as argatroban may prove superior to therapeutic heparins in these situations. Our data also illuminate the need for active surveillance using both D-dimers and fibrinogen in SARS-CoV-2 infection. D-dimer levels declined but remained

significantly elevated in all 5 of our cases after initiation of argatroban, while fibrinogen levels normalized. The fact that ultrafiltration leads to concentration of fibrinogen on dialyzer capillaries [8] may explain the greater sensitivity of fibrinogen over D-dimer levels to changes in circuit thrombosis.

What might account for the clinical superiority of a direct thrombin inhibitor in COVID-19? Suppression of ATIII represents one possibility, as argatroban works independently of ATIII [10]. A recent cohort of 10 patients with severe COVID-19, 9 of whom had confirmed thrombosis on hospitalization despite use of prophylactic LMWH, were successfully treated with argatroban [10]. None had circuit thrombosis with CRRT or ECMO while on this therapy. The investigators suggested that heparin resistance was a consequence of low ATIII levels in their cohort, which ranged from 19 to 49 IU/dl, as levels ≥50 IU/dl are considered necessary to achieve an anticoagulant effect with heparin [10]. However, it appears that ATIII was measured while these patients were on heparin. Heparin lowers levels of detectable ATIII antigen and activity detection by ~30%, a phenomenon thought secondary to accelerated clearance, while having no impact on its anticoagulant activity [11]. In fact, a large case series found that ATIII levels rarely fall below 80% in COVID-19, regardless of disease severity [12]. In the two patients from our cohort for whom ATIII levels were measured, both were >50%: Case 4 at 92% (off UFH), and Case 3 at 58% (measured during UFH use).

Changes in ATIII function in vivo in severe COVID-19 remain a possibility, however, as it can be inactivated by metalloproteinases and coagulation proteases, both elevated in the inflammatory milieu of COVID-19,[4,5] that cleave its catalytic domain without reducing protein levels [13]. In addition, abnormalities in fibrinolysis may be involved. There is a delicate balance between host coagulation and fibrinolytic pathways. SARS-CoV infection of mice can overwhelm pro-fibrinolytic signaling via the urokinase pathway [14]. A thromboelastography study similarly documented abnormalities of fibrinolysis in severe COVID-19 [15]. Argatroban, unlike heparin, can enhance fibrinolysis by differential inhibition of thrombin-mediated activation of thrombin activatable fibrinolysis inhibitor (TAFI) and a decreased, though persistent, activation of factor XIII [16]. Limitations of this study include the small number of subjects evaluasted, arguing for the need of a controlled clinical trial of direct thrombin inhibitors in the setting of persistent clotting occurring among COVID-19 patients receiving standard prophylactic or therapeutic heparin-based regimens.

Authors' contributions

All three authors contributed to study design and data analysis. Patient data were collected by M. S. and J.L. J.L. wrote the paper, which has been reviewed by all authors.

Declaration of competing interest

JL has received grants and honoraria from Alexion, Inc. and Omeros, Inc., manufacturers of anti-complement drugs. The remaining authors declare no competing financial interests.

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